ELECTRON MICROSCOPIC ATLAS
OF NORMAL AND LEUKEMIC HUMAN BLOOD

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To

CHARLES MAYO GOSS

scholar teacher friend

PREFACE

Since the preparation of our first blood specimens more than two years ago we have depended on cooperation and help from many sources The chief financial support of the laboratory has been provided by the United States Public Health Service under Grant H 1663 The RCA EML electron microscope on which most of the electron micrographs were taken was purchased with funds from this grant A substantial part of the running expenses of the laboratory including microscope maintenance was also derived from this source Supplementary support for the laboratory has been generously granted by the Lilly Research Laboratories Annual grants from this source have provided a technician s salary and incidental running expenses Particular thanks are extended to them for a special grant of $2000 supplementary to their annual contribution which was given in recognition of our need for added funds to meet the extraordinary expenses incident to the preparation of this book During the preparation of this book one of us (JAF) was a Public Health Training Fellow under Grant HTS-5111

The Charity Hospital of Louisiana in New Orleans maintains an RCA EMU 2 electron microscope for the use of interested scientific personnel in the New Orleans area this instrument was used in the early stages of the project An RCA EMU 3 electron microscope in the Pathology Department at Louisiana State University was also used

through the courtesy of Dr Henry C McGill His light micrography apparatus was used to take the light micrographs of the leukemias Dr R A Simmons head of the Department of Public Health and Preventive Medicine took the light micrographs of the normal blood cells The International model PR 2 refrigerated centrifuge in which the blood samples were spun down prior to fixation was made available to us through the courtesy of Dr Ernest Bueding of the Department of Pharmacology of this school

Dr Monroe S Samuels of the Department of Pathology in this school and in Charity Hospital cooperated in obtaining blood samples assisted with taking the light micrographs and made the clinical diag noses Without his help in obtaining samples and his advice in inter pretative problems this undertaking would have been extraordinarily difficult Dr Ralph M Hartwell of Hotel Dieu cooperated in obtaining blood samples from certain patients Dr Esther E Anderson of the Louisiana State University Department of Pediatrics assisted with the clinical interpretation of the stem cell leukemias Marjorie Ledoux of the Louisiana State University medical library helped prepare the reference lists The cooperation of Rita Bondio of the Charity Hospital record library in obtaining clinical charts for study is appreciated

In our own laboratory the sections for electron microscopy were cut by Max R Clevenger who also performed general technical work Lucien C Caro and later Robert E Druce maintained the electron microscopes in the fine working order necessary to obtain the electron micrographs Many of the final prints used for engraving were made by Peter Gregorio Louis LaScola of the Louisiana State University De partment of Medical Illustration lettered the prints Don Alvarado head of the Department of Medical Illustration made the drawing on page 6

Much of the uninterrupted progress of the work has been due to the liberal policies of academic freedom expressed by Dr Charles M Goss head of the Department of Anatomy and by Dr Wm W Frye Dean of Louisiana State University School of Medicine

F N L
J A F

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INTRODUCTION

During the past few years excellent progress has been made in the development and use of preparatory techniques for biological electron microscopy Plastic embedding [5] buffered osmium tetroxide fixation [3] glass knives [1] and a new and improved ultramicrotome [4] have all combined their merits to make high-quality preparations routinely possible All the soft biological tissues are now amenable to analysis at the electron level of magnification In keeping with these advances an electron microscopic investigation of ultrastructure in the leukocytes of normal and leukemic human blood was undertaken along the following lines

TECHNIQUE

During the summer of 1955 James A Freeman developed a technique for the collection of samples of human blood for electron microscopic study [2] The method was designed to involve the least possible technical manipulation of the blood sample both before and after fixation All instruments were coated with nonwetting agents and the blood was refrigerated to prevent or delay clotting The sample was centrifuged to concentrate the buffy coat thereby obtaining minimal contamination by

erythrocytes No anticoagulant or any other foreign substance was added to the blood prior to fixation

About 6 to 7 cc of blood was obtained by venipuncture either (*a*) by withdrawal with a 10-cc syringe fitted with a 20 gauge needle and transference to a 10-cc Lusteroid centrifuge tube (International) precooled to 5 to 10°C or (*b*) by needle drip directly into the tube The syringe had been previously silicon coated with Dow Corning 200 2 per cent in CCl_4 by immersion and baking for ½ to 1 hour at 450 to 550°C The needle had been coated with 10 per cent aqueous Armour Monocote [tris (2 hydroxyethyldodecyl) NH_4Cl] by immersion draining and air drying The ice-cooled sample was then centrifuged at 1500 rpm for 15 minutes at 0°C (relative centrifugal force = 265 International model PR 2 refrigerated angle head) The buffy coat was aspirated with a silicon-coated pipette and transferred to a glass tube containing 5 cc of 1 per cent Veronal buffered (pH = 7 4) OsO_4 at 5 to 10 C [3] It was fixed for ½ or 1 hour usually the former Between the successive steps (½ to 1 hour) of fixation dehydration and methacrylate infiltration the specimen was centrifuged for 1 to 1½ minutes at 1500 rpm (relative centrifugal force = 385 Clay Adams Safeguard) in glass tubes (alcohol dissolves Lusteroid!) After each centrifugation the supernatant fluid was decanted the next fluid added and the tube manually agitated to produce a suspension The last methacrylate suspension (6 parts *n* butyl 1 part methyl) was permitted to settle by gravity in 00 gelatin capsules for ½ to 1 hour to avoid close packing This also eliminated bubble formation during polymerization which was performed overnight at 47°C Sections were cut on a Porter Blum microtome using a glass knife and were mounted on copper grids covered by Formvar or carbon membranes Three RCA electron microscopes were used for viewing and photography an EMU 2 an EML 1 B and an EMU 3 The micrographs were taken on 2 by 10 in or 3¼ by 4 in Kodak lantern slide medium plates and were printed by projection enlargement Neither prints nor negatives were retouched

MATERIALS

The normal blood samples used in this study were obtained from volunteer donors The leukemic blood was obtained from patients at the Charity Hospital of Louisiana in New Orleans They represent a typical group of leukemia patients coming into a 3000 bed hospital over a period of a little over 2 years At the same time that the blood samples were obtained for electron microscopy smears were prepared stained by Wright s method and studied under the light microscope The pertinent data on the blood samples are given in the table on pages 4 and 5

CHARACTERISTICS OF ELECTRON MICROGRAPHS

Electron micrographs depict cellular morphology in much greater detail than the finest light micrographs Observers familiar with the ap

pearance of tissues in light microscopy experience no difficulty in recog nizing most cellular structures in electron micrographs But there are fundamental and sometimes quite significant differences between the two kinds of preparation Certain inaccuracies may arise from incautious interpretation of electron micrographs of leukocytes by those primarily familiar with the hematology of light microscopy Since these misinter pretations can be avoided by the use of a few simple and easily applied principles the significant differences between light and electron micro graphs are discussed below in the order of their importance

The most important difference between the preparations of light and electron microscopy is traceable to the actual amount of tissue present In smear preparations those most commonly used in usual hematologic preparations entire cells are present All the formed elements of the cell which have been preserved by the technique are available for examina tion But in electron microscopy very thin sections of cells averaging $1/_{0}$ to $1/_{30}$ μ thick are used A $1/_{0}$ μ section through the middle of a rounded up cell 10 μ in diameter contains only 3/4 of 1 per cent of the total volume of the cell This presents a very revealing picture of the formed elements contained within that particular section But compar able information about the remainder of the cell would require 133 more sections in serial order an obvious impracticality The visualization of any structure in a particular section may be accepted as proof of its presence in a cell but its absence is not evidence that the cell does not possess such a structure Thus the thin sections of electron microscopy impose an essentially statistical approach to the interpretation of cellu lar ultrastructure Many sections from a number of cells the larger the better must be studied to form an idea of their structural organization

Another feature of importance traceable to the thin sections of elec tron microscopy is the fact that the plane of section through any cell is haphazard It may pass centrally or peripherally by chance and in any direction The figure on page 6 illustrates some of the ambiguities arising from this circumstance In this figure each one of the four hypo thetical planes of section results in a section quite different from the rest although all are derived from the same cell

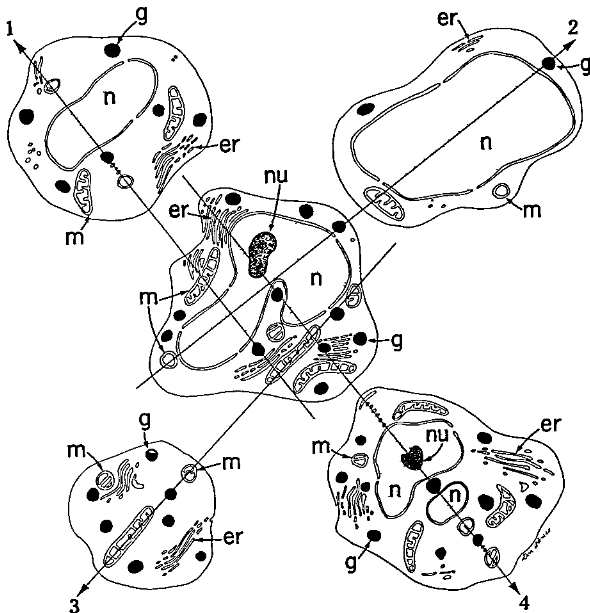
It is well to be aware of certain other ways in which electron micro graphs differ from those of light microscopy although they are of less critical significance The densities of light micrographs are traceable to variable light transmission and chromatic factors which are familiar to all But the densities in an electron micrograph are functions of the atomic mass of the elements present in any particular part of the speci men When the atomic mass is high as in areas occupied by concentra tions of heavy metals the density is high and the area is dark Wherever the atomic mass is low as in parts of the specimen populated by elements of low atomic weight corresponding areas of the micrograph are light All the electron micrographs in this volume were made from tissues fixed with osmium tetroxide and the varying densities in them are largely expressions of local distributions of osmium atoms Osmium with a specific gravity of 22 5 is the most dense of all the elements This

Case No	Sex	Age	Clinical Diagnosis	WBC Count	Peripheral Blood Picture	Therapy before Specimen Obtained
1a	Male	22	Normal	6 500	Normal	None
1b	Male	22	Normal	7 200	Normal	None
1c	Male	22	Normal	6 800	Normal	None
1d	Male	22	Normal	8 100	Normal	None
1e	Male	23	Normal	—	Normal	None
1f	Male	23	Normal	7 400	Normal	None
1g	Male	24	Normal	6 700	Normal	None
2a	Male	23	Normal	7 600	Normal	None
2b	Male	23	Normal	7 500	Normal	None
3a	Male	20	Normal	7 400	Normal	None
3b	Male	21	Normal	—	Normal basophils 6%	None
4	Male	22	Normal	7 000	Normal	None
5	Female	16	Normal	8 400	Normal	None
6	Male	23	Normal	8 200	Normal	None
7	Male	46	Normal	—	Normal	None
8	Male	56	Normal	—	Normal	None
9	Male	26	Normal	—	Normal basophils 4%	None
10	Female	15	Undiagnosed illness	15 500	Eosinophils 50%	None
11	Female	58	Undiagnosed illness	—	Eosinophils 57%	None
12	Male	3	Pneumonitis	125 000	Eosinophils 85%	None
13	Male	47	Carcinoma of esophagus	11 500	Eosinophils 40% basophils 4%	None
14	Female	62	Chronic myelogenous leukemia	86 200	——	None
15	Female	60	Chronic myelogenous leukemia	235 000	Metamyelocytes 20% myelocytes 11% blasts 5%	None
16	Female	48	Chronic myelogenous leukemia	37 000	Metamyelocytes 8% myelocytes 6% blasts 4%	None
17	Female	63	Chronic myelogenous leukemia	15 000	Metamyelocytes 28% myelocytes 11% blasts 4%	None
18a	Female	52	Subacute myelogenous leukemia	397 000	Metamyelocytes 15% myelocytes 20% blasts 10%	None
18b	Female	52	Subacute myelogenous leukemia	184 000	Metamyelocytes 19% myelocytes 13% blasts 3%	Myleran 2 mg/day for 4 days x ray 50 r to spleen
19	Male	38	Subacute myelogenous leukemia	81 000	Metamyelocytes 20% myelocytes 20% blasts 10%	X ray twice to spleen and three times whole body irradiation over 1¼ year period Myleran 6 mg/day for 7 days then 4 mg/day for 21 days
20	Male	52	Acute myelogenous leukemia	287 000	Metamyelocytes 6% myelocytes 8% blasts 65%	None
21	Male	5	Acute stem cell leukemia	1 850	Stem cells 20%	None
22	Female	7	Acute stem cell leukemia	35 000	Stem cells 80%	6 Mercaptopurine 40 mg every 2 days for 4 days
23	Male	3	Acute stem cell leukemia	1 250	Stem cells 14%	6 Mercaptopurine 37 mg every 2 days (last dose 2 days prior to specimen)
24	Male	63	Chronic lymphocytic leukemia	30 000	Mature lymphocytes 95%	None
25	Female	49	Chronic lymphocytic leukemia	5 300	Mature lymphocytes 33%	None

Case No	Sex	Age	Clinical Diagnosis	WBC Count	Peripheral Blood Picture	Therapy before Specimen Obtained
26	Male	70	Chronic lymphocytic leukemia	143 000	Lymphocytes 95% most immature	X ray 150 r to spleen for 2 days (last dose 2 days prior to speci men)
27	Female	68	Chronic lymphocytic leukemia	28 400	Mature lymphocytes 70% immature 10%	None
28	Female	79	Chronic lymphocytic leukemia	52 000	Mature lymphocytes 88%	None
29	Male	77	Chronic lymphocytic leukemia	239 000	Lymphocytes 95% most immature	None
30	Female	68	Subacute lymphocytic leukemia	25 000	Mature lymphocytes 64% immature 11%	None
31a	Male	16	Acute lymphocytic leukemia	520 000	Immature lymphocytes 98%	None
31b	Male	16	Acute lymphocytic leukemia	13 700	Immature lymphocytes 73%	Purinethol 125 mg/day for 4 days
32	Male	—	Acute lymphocytic leukemia	170 000	—	None
33	Female	63	Acute lymphocytic leukemia	25 000	Mature lymphocytes 12% immature 80%	None
34a	Male	40	Acute monocytic leukemia	13 500	Blasts 83% many with Auer bodies	None
34b	Male	40	Acute monocytic leukemia	18 200	Blasts 50%	None
35a	Female	53	Acute monocytic leukemia	6 100	Blasts 47%	None
35b	Female	53	Acute monocytic leukemia	64 000	Blasts 90%	Blood transfusions
35c	Female	53	Acute monocytic leukemia	63 400	—	6 Mercaptopurine 50 mg/day for 3 days then 50 mg every two days for 2 weeks (till expiration)
35d	Female	53	Acute monocytic leukemia	57 600	—	
35e	Female	53	Acute monocytic leukemia	120 000	—	
36	Female	81	Multiple myeloma	—	Normocytic normochromic anemia	None

circumstance combined with the fact that it has strong affinity for some parts of the tissue and scarcely any at all for others produces a wide range of densities Fortunately the density patterns in electron micro graphs after osmium tetroxide fixation so closely parallel those of ordi nary black and white light micrographs that little interpretative difficulty is encountered But the chromatic staining so useful in light microscopy cannot be transferred to electron microscopy with profit The various dyes have much the same chemical elements in their make up and hence similar atomic masses They would therefore not be distinguishable from one another in an electron micrograph Osmium tetroxide in effect stains as it fixes and the result is essentially immutable But the wide range of resultant densities and the higher resolution of the electron mi croscope more than counterbalance any limitation set by the present lack of *selective staining*

It is notable that most of the cells in the illustrations are oval rather than round in their outlines In the low power fields containing many cells their long axes are in general oriented in the same direction This is due to an unavoidable compression of the section during cutting



Relationship of the plane of a thin section through a cell to the appearance of an electron mi rograph of that section In the center is a typical cell containing a nucleus (*n*) nucleolus (*nu*) mitochondria (*m*) endoplasmic reticulum (*er*) and granules (*g*) Each of the figures in the four corners numbered 1 to 4 illustrates the appearance of an electron micrograph of a section of the center cell which passed through it in the position of the arrow The thickness of the arrow is drawn roughly to scale representing section thickness relative to cell size Note the great difference in general appearanc o the four resultant sections This occurs because only the structures contained in the thickness of the section are depicted in the final micrograph

Its effect is not damaging and may be ignored except where size measurements are of critical importance The long axis of the cell not having been subjected to compression is the proper direction for taking measurements

GENERAL PROCEDURE

Visual inspection of preparations in the microscope itself is adequate for summary judgment of specimen quality and selection of potentially interesting fields But serious study of the specimens as visually observed in the microscope itself is limited by a number of significant factors The softening effect of the screen the dimness of the image at higher magnifications and the known presence of details in the image too small to be seen by the operator s eye all require that a permanent record be made for examination under more favorable conditions Therefore any fields which appear to be worthy of closer examination are photographed The plates are developed immediately and viewed for quality Subsequently all satisfactory negatives are printed enlarged four diameters These file prints are the material that is subjected to serious analysis and study From time to time each category of file prints is supplemented by new micrographs searched out from fresh preparations of the same specimens to determine the validity and frequency of structural characteristics revealed in the original set Finally representative fields from each dossier of file prints are chosen for quality variety and general value These are worked up to serve as published illustrations

In the course of this investigation more than 4000 separate negatives were obtained Although economy in choice of fields for publication is commendable the use of a fairly large number of illustrations is desirable for a number of reasons The larger the number of illustrations the closer the reader approaches to the conditions under which the original study and analysis were made that is to the study of file prints The characteristics of electron microscopic preparations cited above are such that they encourage if not force a statistical approach to interpretation For example the size and shape of nuclei become evident only if a fairly representative number of cells has been observed Furthermore there is considerable spontaneous variation in the general appearance of specimens obtained from the same source and prepared by the same method even though no technical flaws are present A variety of illustrations should help the reader appreciate such variations So much new detail is revealed by electron micrographs that it is frequently impractical to describe and call attention to all points of interest in a single micrograph since overlengthy text and crowded labeling would result

The text is primarily descriptive Interpretation has been restricted almost entirely to identification of cell types and readily recognizable structures The terminology has been kept as simple as possible with preference for basic science terms in general use Wherever synonymous

terms are widely used both have been cited in the short text preceding the section One has been chosen arbitrarily and used in the facing page descriptions Reference to previously published work has been re stricted to the introductory text preceding each section, with the refer ences cited at the end of that text It has been cut to a minimum with citation of only the most significant contributions

In keeping with its primary significance, the section on normal blood is the largest one It is followed by descriptions of the granulocytic and myeloblastic leukemias Both these sections are quite extensive because of the wide variety of cell types represented Together they cover the development of the granulocytic series from its forerunner the myelo blast to mature granulocytes The stem cell leukemias are next consid ered In untreated cases the stem cells cannot be distinguished from the myeloblasts of a myeloblastic leukemia *Considerable attention has* been paid in this section to the development during treatment of a case of stem cell leukemia of an unknown and apparently mature cell type which probably arose as a result of the therapy Lymphocytic leukemia, although clinically one of the most common types is covered in a short section This is because only the lymphocytic series of development is represented and the transition from lymphoblast to lymphocyte pre sents very few visible changes in electron microscopy The section on monoblastic leukemia describes this developmental series with special attention paid to Auer bodies and the fibrillar formation often seen in these cells A section on plasma cells has been included because these cells are present in normal blood (although rarely) and are common in eosinophilia A few occur in certain of the leukemias In multiple myeloma they may be so numerous in peripheral blood that this condi tion is then called a plasma cell leukemia The book closes with a very brief section on members of the erythrocytic series that are occasion ally present in the leukemias studied to avoid confusion with cells of the leukocytic series

A number of light micrographs of Wright stained peripheral blood smears of both normal and leukemic human blood have been included in addition to the electron micrographs These are all shown at the top of the left hand page for comparison with electron micrographs on the right hand page All illustrations on right hand pages are electron micrographs

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NORMAL BLOOD

This section presents the normal electron morphology of human leuko cytes and platelets The micrographs were chosen to cover the subject in as concise and comprehensive a manner as possible They are system atically arranged so as to present to the reader a progressively more detailed concept of cellular ultrastructure A group of mixed fields containing different cell types is followed by a more thorough descrip tion of each The section closes with an account of the general electron cytology of leukocytes

The illustrations in the first group (General Fields) were chosen for their variety These should introduce the reader to the obvious differ ences between the various cell types and establish a general familiarity with each The structural features of each cell type are dealt with only briefly a more detailed analysis being presented in subsequent pages to which reference may be made for greater detail

Electron microscopic studies of normal blood cells have been made by Bernhard et al [1 2] Bessis [3 4] De Robertis [6] Di Mayorca et al [13] Grey and Biesele [8] Kautz and De Marsh [10 11] Miller [14] and others [24 25 26] In general the results of this study so closely parallel the findings of these authors that it would be super fluous to cite direct comparisons in each case However a few contro versial points of interpretation have arisen Wherever these have oc

curred specific reference has been made to other publications in the fol lowing text

The neutrophils are the first cell type considered They present a coherent and easily recognizable cell type The specific granules the polymorphous nucleus and the clearly differentiated nucleoplasm in characteristic patterns readily identify these cells Only in occasional ambiguous cases could their identity be confused with any of the other leukocytes

The next section describes eosinophils The ultrastructure of the spe cific granules characterizes this cell type However it is entirely pos sible that some cells of normal human blood now routinely identified in electron microscopy as eosinophils may in fact be basophils A clear distinction between eosinophils and basophils cannot be made at the present time This is because positively identified basophils are not found in blood prepared by this technique It is possible that deceptive similarity in the ultrastructure of the granules of these two types of cells may be responsible for this Since the question is unsolved the following discussion seems to be merited

Eosinophilic granules are generally understood to possess a character istic internal ultrastructure [8 14 19 30] examples of which are illus trated on pages 61 and 63 During our work cells containing this type of granule were interpreted to be eosinophils Basophils on the other hand are understood to possess granules that are homogeneous [8 19 30] Therefore search was made in our preparations for granu locytes with large dense homogeneous granules Since basophils con stitute only ½ to 1 per cent of the total leukocyte count it was expected that this search would be difficult But cells answering this description were not discovered in the examination of a large number of specimens during the course of which more than 4000 electron micrographs of different cell types were taken All these specimens should have con tained normal basophils Special cases were therefore examined Normal blood from two individuals one having a 6 per cent basophil count and the other a 3 to 4 per cent basophil count were examined The blood of a hospital patient with a 40 per cent eosinophil and 4 per cent baso phil count was also examined The results were negative in all cases although eosinophils were readily recognizable in all of them In the last case at least 200 cells with internal ultrastructure in their granules were observed and these were interpreted to be eosinophils

Unfortunate operation of statistical probability cannot be entirely ruled out as the cause of the negative results but the extensiveness of the search renders this extremely improbable The possibility that the granules of human basophils may possess an ultrastructure should be considered All the electron microscopic studies on basophilic granules have been made on experimental animals and ultrastructure in these granules has been reported by at least one worker [22] Human baso phils with homogeneous granules have not to our knowledge been re ported The developing cells of the human leukemias unfortunately offer no solution The later developmental stages the myelocytes and meta

myelocytes have granules of the eosinophilic type with internal ultra structure But the earlier forms the promyelocytes and some myeloctyes have structureless granules of the basophilic type There is strong suggestion of developing internal ultrastructure in the later forms as reported by Pease [22] It is possible that all the cells in our prepara tions are members of the eosinophilic series the earlier forms possess ing granules that are still homogeneous However if human basophils actually possess granules containing ultrastructure similar to that of eosinophilic granules both types of cell may be represented

Selective destruction of basophilic forms by the technique is a possi bility that also must be considered However fragmentation during centrifugation cannot account for their absence since basophils have been identified in Wright stained smears taken from the centrifuged buffy coat immediately before fixation Good preservation of basophils and mast cells in animal tissues prepared by the same techniques of fixation dehydration and embedding make it unlikely that selective destruction has occurred during these processes No fragments of damaged cells have ever been observed in our preparations The weight of evidence favors the belief that basophilic granules possess an ultra structure deceptively similar to that of eosinophilic granules

In view of these circumstances no attempt has been made to illus trate basophils The section of eosinophils illustrates cells chosen for the classic features of this cell type However one structural feature the elements called *basophilic bodies* merits special notice These bodies were so numerous in a case of eosinophilia (Case 12) that they were noticed in Wright stained smears under the light microscope Their identity and prevalence were confirmed by electron microscopic exami nation They are present in normal eosinophils and have been ob served by others although interpreted by one investigator to be the granules of basophils [13]

The sections on lymphocytes and monocytes both describe and com pare these cell types The distinction between them is perhaps not so difficult in electron microscopy as in light microscopy The lymphocytes have large mitochondria sparse endoplasmic reticulum and differenti ated nucleoplasm whose two densities light and dark tend to be pat ternless rather than systematically localized as in other cell types The monocytes have numerous small mitochondria which are mostly circu lar very numerous small round or oval profiles of endoplasmic reticu lum generally speckled cytoplasm and differentiated nucleoplasm whose dark component tends to form an irregular band inside the nuclear membrane However a clear distinction between a monocyte and a lymphocyte cannot always be made An example of this is pre sented on page 87

Blood platelets are readily recognized in these preparations and are illustrated on pages 89 to 101 In general they conform to light micro scope descriptions except that unusually large ones (pages 89 101) are surprisingly common These have been described in light microscopy as *giant platelets* but have received very little attention In general the

electron microscopy of blood platelets suggests that they are cyto plasmic fragments containing granules mitochondria, and whatever other elements might be expected to be present in any random piece of cytoplasm of comparable size The chromomere and hyalomere of light microscopy are readily recognized The plasma membrane is quite dense but the density of the other components seems to vary greatly in specimens that appear to be well fixed

A section on general electron cytology as it appears in blood cells has been included Agranulocytes demonstrate most of the illustrated structures to best advantage Definitive studies on the electron micros copy of these fundamental cellular structures have been made as fol lows mitochondria [12 15 16 18, 27] nuclear pores [7 21, 29] endoplasmic reticulum [18 19 20 23 31] Golgi zone [5 9] Palade granules [17] *In general the electron cytology of blood presents no* very special features in comparison with other tissue cells except for the specific granules which are described in detail under their particu lar cell types

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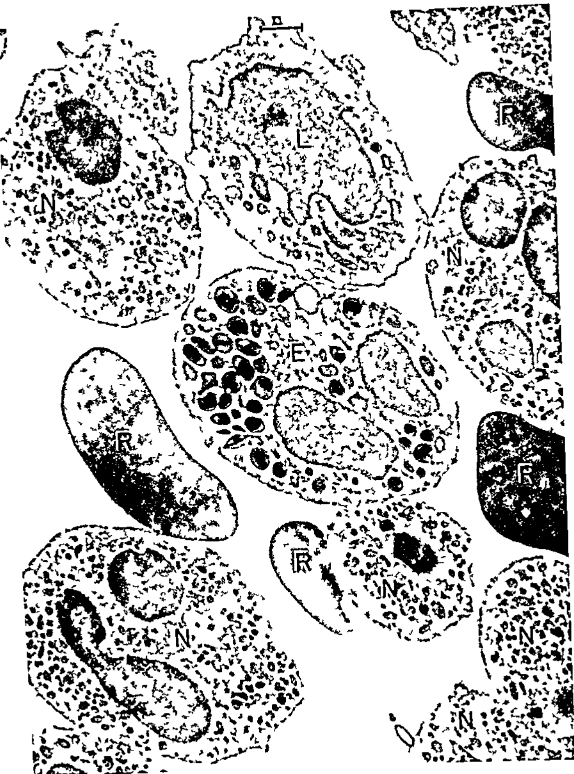
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31 Weiss J The ergastoplasm Its fine structure and relation to protein synthesis as studied with the electron microscope in the pancreas of the Swiss albino mouse *J Exper Med* 98(6) 607–618 1953



NORMAL BLOOD MICROGRAPHS

(Case 1f) 11 000 X

Neutrophils (N) red blood cells (R) eosinophil (E) and lymphocyte (L) The chief distinguishing features of the neutrophil are the small moderately dense specific granules and the polymorphous nucleus more than one lobe of which usually appears in the plane of section Eosinophils can be identified because of the many large specific granules which have a distinct internal ultrastructure Lymphocytes have cytoplasm that is largely clear with only a small amount of endoplasmic reticulum large round or rod shaped mitochondria and a nuclear density pattern that is characteristic of this cell type Detailed criteria for identification of cell types may be found in the sections following on neutrophils eosinophils and lymphocytes


L
R
N
N
E
R
R
R
N
N
N
N

(Case If) 8000 X

Neutrophils (N) and two eosinophils (E) The polymorphous nuclei of the neutrophils often show several separate lobes because the thin connecting strands are usually not included in the plane of section The specific neutrophilic granules (*ng*) in the cytoplasm are conspicuous The eosinophilic granules (*eg*) are clearly distinguishable from the neutrophilic ones They are large and possess an internal ultrastructure of their own (pages 61 and 63) In the sectioned preparations of electron microscopy certain basophilic bodies (*bb*) are conspicuous in normal eosinophils These are of uniform density without discrete internal ultrastructure They probably correspond to certain basophilic inclusions recognized in light microscopy



N
ng
N
N
E
bb
N
N
bb
ng
eg
E
bb
N
N

(Case 1f) 14 000 X

Neutrophils and one eosinophil (*E*) Two distinct densities one light and the other dark are evident in the nuclei of the neutrophils The darker of the two is located peripherally constituting a thick band subjacent to the nuclear membrane The central area of the nuclear lobe is occupied chiefly by the lighter density but occasionally the darker nucleoplasm (*nd*) invaginates it There is also an occasional extension of the lighter density (*nl*) toward the nuclear membrane In general these densities correspond to the chromatin pattern characteristic of these nuclei in light microscopy *Note that the eosinophilic* nucleus has a similar density pattern

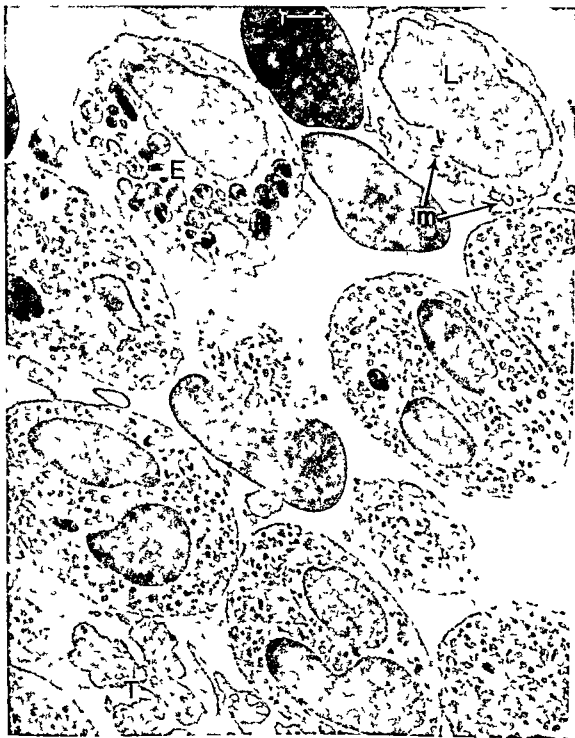
The small evaginations of the plasma membrane (*pm*) are very common in these preparations and may occur in any type of cell The structure at *T* is a blood platelet (pages 88 to 101)


E
pm
pm
T
nl
nl
nd
pm
nd

(Case 1f) 14 000 X

Neutrophils and one eosinophil (*E*) Two distinct densities one light and the other dark are evident in the nuclei of the neutrophils The darker of the two is located peripherally constituting a thick band subjacent to the nuclear membrane The central area of the nuclear lobe is occupied chiefly by the lighter density but occasionally the darker nucleoplasm (*nd*) invaginates it There is also an occasional extension of the lighter density (*nl*) toward the nuclear membrane In general these densities correspond to the chromatin pattern characteristic of these nuclei in light microscopy Note that the eosinophilic nucleus has a similar density pattern

The small evaginations of the plasma membrane (*pm*) are very common in these preparations and may occur in any type of cell The structure at *T* is a blood platelet (pages 88 to 101)


L
E
m
T

(Case 1f) 11 000 X

Mixed field An eosinophil (E) and a lymphocyte (L) occupy the upper part of this field The remainder of the leukocytes are typical neutrophils A small group of blood platelets may be seen in the lower left hand corner (T)

The density pattern in the nuclei of the neutrophils is typical It may be compared to the pattern in the nucleus of the eosinophil (E) which is much the same In general the eosinophils have more of the denser component of the nucleoplasm in the center of the nuclear lobes than the neutrophils But the density patterns in the two resemble each other so closely that they are not useful as a distinguishing characteristic

The lymphocyte (L) has cytoplasm that is clear relative to that of the granulocytes The mitochondria (m) in this particular cell are somewhat smaller than usual The nuclear density is typical There is not a clear patternization of two densities as there is in the nuclei of neutrophils and eosinophils



M
m
M
N
m
L
M
er
m
er
N
m
L
N
N

(Case If) 11 000 X

Three different kinds of leukocytes with deceptively similar general appearance Neutrophils are at *N* lymphocytes at *L* and monocytes at *M*

The neutrophils may be identified chiefly by the presence of the small medium dense specific granules and in fortuitous cases by the pattern of nuclear densities The lymphocytes have large mitochondria (*m*) many of which are rod shaped The nucleoplasm does not usually form an organized pattern of dark and light components as in neutrophils eosinophils and monocytes (pages 45 57 79) The monocytes (*M*) possess small round mitochondria (*m*) although rod shaped ones are sometimes observed The nucleoplasm clearly shows two densities the darker of the two being subjacent to the nuclear membrane This pattern resembles that of neutrophils but the dense component is relatively thinner in the monocytes A further aid to the identification of monocytes is the relative abundance of endoplasmic reticulum (*er*) as compared to that of lymphocytes and neutrophils This reticulum appears as clear round or oval vacuoles scattered through the cytoplasm

The criteria for identification of these cell types are described in more detail elsewhere (under Neutrophils Lymphocytes and Monocytes)

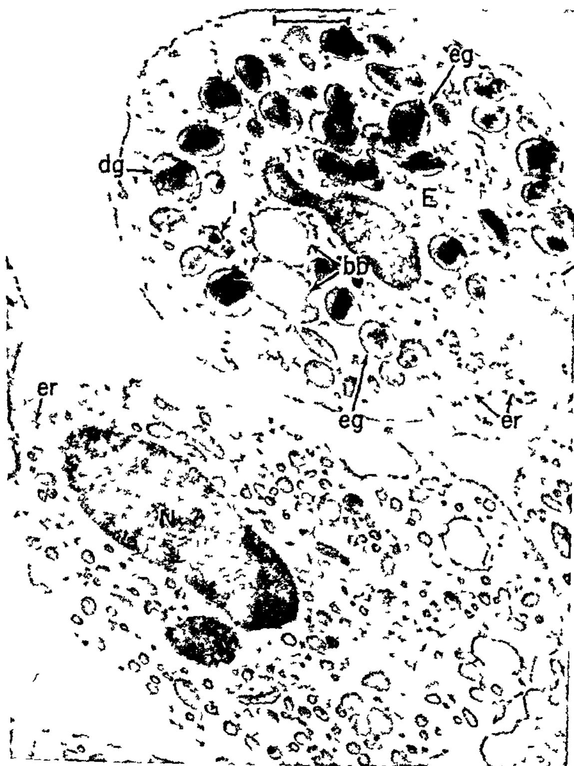

bb
ng
N
eg
bb
E
bb

(Case 1f) 21 000 X

Eosinophil (E) and neutrophil (N) The eosinophilic granules (*eg*) are clearly evident and there are five evident basophilic bodies (*bb*) These are typical of their kind with a structureless matrix of medium density surrounded by an irregularly deckled margin of distinctly higher density (pages 61 and 63)

The neutrophilic granules (*ng*) show no unusual features In neutrophils there is often an area of cytoplasm free of specific granules immediately surrounding the nucleus It is evident in this cell especially around the lower lobe of the nucleus But fairly sizable areas of the cytoplasm elsewhere may be free of specific granules

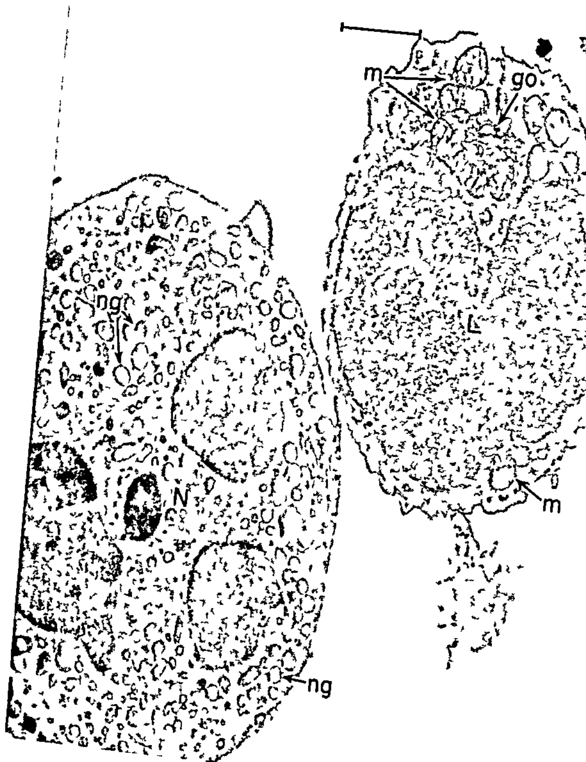
Note the close similarity of nucleoplasmic density and pattern in these two cells Neutrophils and eosinophils cannot be distinguished from each other on the basis of this feature of their ultrastructure


eg
dg
E
bb
er
eg
er
N

(Case 1f) 18 000 X

Eosinophil (E) and neutrophil (N) The eosinophilic granules (*eg*) show the two densities typical of their internal ultrastructure There are two basophilic bodies present (*bb*) The inclusion at *i* has irregular contours and variable internal densities which suggest a broken up appearance It may be compared with the degenerating granule at *dg* Such structures while not numerous are observed in even the best preserved cells A characteristic area of endoplasmic reticulum is seen at *er*

The neutrophil (*N*) is typical in all respects Note the variation in the size of the specific granules which varies from less than 0 2 μ to nearly 1 μ The nuclear densities in this cell are characteristic The endoplasmic reticulum is represented only by a few small isolated vacuoles (*er*)


m
go
ng
N
L
m
ng

(Case Ib) 21 000 X

Lymphocyte (L) and neutrophil (N) The mitochondria (*m*) in the lymphocyte are typical of those of lymphocytes in general The round and oval ones are 0 5 μ in their largest measurement and the rod shaped one is about 1 2 μ long The Golgi zone (*go*) is visible

The neutrophilic granules (*ng*) are typical in their distribution and size Because the section is somewhat thinner than usual the dark external membrane contrasts well with the homogeneous moderately dense matrix


m
go
m
L
N
er
m
er
m
er
M
N

(Case 1f) 20 000 X

Lymphocyte (L) portions of two neutrophils (N) and monocyte (M) The lymphocyte is typical containing large mitochondria (*m*) which are round or rod shaped Endoplasmic reticulum (*er*) is very sparse The Golgi zone (*go*) is visible The lack of coherent patternization of the two densities in the nucleoplasm is characteristic of lymphocytes

The cytoplasm of the monocyte contains an abundance of small round mitochrondria (*m*) and a typically well-developed system of endoplasmic reticulum The denser component of the nucleoplasm is largely subjacent to the nuclear membrane a characteristic of mono cytes

The over all density of the monocytic cytoplasm bears a superficial resemblance to that of the neutrophils


m
go
m
L
er
N
m
er
m
er
M
N

GENERAL FIELDS

(Case 1f) 8000 X

Typical neutrophils and small portion of eosinophil (E) The small neutrophilic granulations of medium density and variable size and shape largely fill the cytoplasm of the neutrophils *In this field both* nuclei and granulations are typical and afford the chief means of identifying the cells as neutrophils The erythrocytes (*R*) are typical


R
E
F
F

(Case 1f) 11 000 X

Four typical neutrophils The thin sections of electron microscopy usually include one or more lobes of the polymorphous nucleus Occasionally one of the thin strands connecting nuclear lobes is visible in the section for a short distance (*ns*) The specific granules (*ns*) are readily recognizable because of their small size and their density which is greater than that of the surrounding cytoplasm They are randomly distributed except for a narrow perinuclear band of relatively clear cytoplasm


ng
ns
ng

1600 X

Light micrograph of neutrophil Its polymorphous nucleus may be compared with the nuclear lobes in the electron micrograph on the facing page Its granules seem to show some variation in size a circumstance confirmed by the electron micrograph

(Case Ia) 25 000 X

Electron micrograph of neutrophil The cell has been cut so that three nuclear lobes appear in the section The two densities characteristic of the nucleoplasm of neutrophils do not show so clearly in this cell as in many others (pages 41 43 45) The neutrophilic granules (*ng*) which vary from the smallest recognizable spheres to ovals and rods which may reach a length of about 0 5 μ are typical Neutrophils are characteristically poor in endoplasmic reticulum (*er*) which appears as small circular profiles having a dark membrane and a light apparently empty center The two oval structures at *m* are mitochondria which are normally found in neutrophils


er
er
ng
m

(Case 1f) 22 000 X

Neutrophil Two nuclear lobes are in the plane of section and one includes a portion of the thin strand (*ns*) connecting adjacent lobes The neutrophilic granules (*ng*) are characteristic in size and distribution Certain of them show to advantage the dark membrane which surrounds the homogeneous moderately dense matrix A few randomly distributed profiles of endoplasmic reticulum (*er*) are visible No *surely identified mitochondria are present in the plane of section* but the oval structure at *m* may be one


ng
er
ns
m
er

(Case If) 22 000 X

Neutrophil with protuberance The specific granules (*ng*) are readily recognizable some with an external dark membrane Among them are granules of the same size and shape containing broken up material of variable density (*dg*) Such grar les may be degenerate but are found even in the best preparations They are somewhat more numerous than usual in this cell

The protuberance in the upper right portion of the cell is unusual since neutrophils prepared by this technique are usually well rounded However it is probably not an expression of pseudopodic activity as it contains its full complement of granules unlike developing pseudo pods (Compare with cell on page 45)


dg
ng
dg
dg
ng

(Case If) 21 000 X

Neutrophil believed to be developing a pseudopod in course of ameboid movement (in direction of large arrow) Clear cytoplasm is involved in its development the specific granules and other formed elements not *yet having flowed into it*

The nuclear lobes of this cell illustrate well the two typical densities of the nucleoplasm The denser of the two (*nd*) is subjacent to the nuclear membrane with the lighter nucleoplasm (*nl*) occupying a more central position It is not unusual for the dense component to extend into the central portion of the lobe or for the lighter com *ponent to extend toward the nuclear membrane (see also page 21)*



Normal Bloo...

(Case 1b) 24 000 X

Neutrophil (N) and group of blood platelets (T) The granules of blood platelets (*tg*) bear a close resemblance to the neutrophilic gran ules (*ng*) Both are of medium density with a structureless matrix which is usually surrounded by a dark membrane A detailed description of platelets will be found on pages 88 to 101

The neutrophil illustrated here presents no unusual features A small amount of Golgi zone (*go*) is present and some mitochondria may be identified (*m*)


fg
T
m
fg
N
m
go
ng

Detail of neutrophilic granules

Upper (Case Ib) 66 000 X

The neutrophilic granules are composed of a moderately dense structureless matrix surrounded by a membrane of higher density The structure having a double membrane (*m*) is probably a mitochondrion The loop of plasma membrane extending from the surface of the cell (*pm*) is characteristic of these preparations (see page 21) The double membrane (*nm*) of the nucleus is characteristic

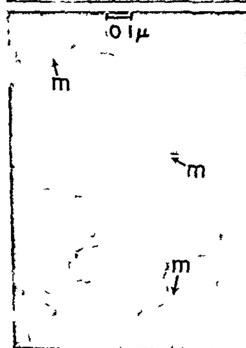
Lower left (Case Ib) 66 000 X

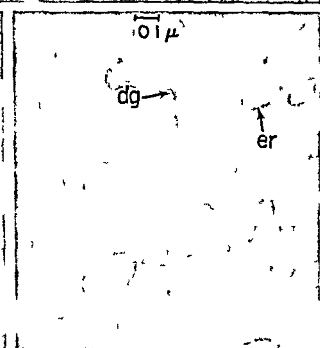
The granules shown here are typical Mitochondria are at *m*

Lower right (Case Ib) 66 000 X

In this field are normal variations in size shape and density of the granules The partially filled granule (*dg*) is representative of many that seem to have a broken up matrix (for illustrations of others see page 43) The vacuole with a clear center (*er*) is apparently a profile of endoplasmic reticulum

0.1 μ
pm
m
nm


0.1 μ
m
m
m


0.1 μ
dg
er

Detail in neutrophilic cytoplasm Mitochondria (*m*) and neutrophilic granules (*ng*) are present in all micrographs

Upper left (Case Ia) 43 000 X

A few profiles of endoplasmic reticulum (*er*) are evident by their light apparently empty centers There are a large number of granular structures (*g*) which may correspond to the *microsome fraction* Compound vacuoles are present (*cv*)

Upper right (Case Ia) 43 000 X

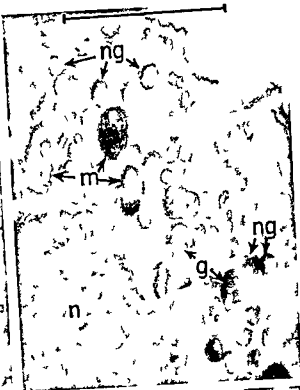
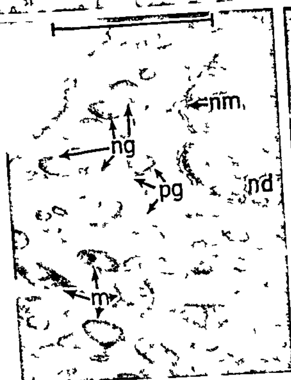
A number of granular structures (*g*) are difficult to identify as representing either mitochondria or neutrophilic granules A portion of the nucleus (*n*) is present

Lower left (Case Ia) 43 000 X

There are some Palade granules (*pg*) present In the nucleus the dense nuclear component (*nd*) is close to the nuclear membrane (*nm*) which shows doubling

Lower right (Case Ia) 43 000 X

In the nucleoplasm the dense component (*nd*) is close to the nuclear membrane A Golgi zone (*go*) is present


ng
g
cv
m
er
ng

ng
m
ng
g
n

nm
ng
pg
nd
m

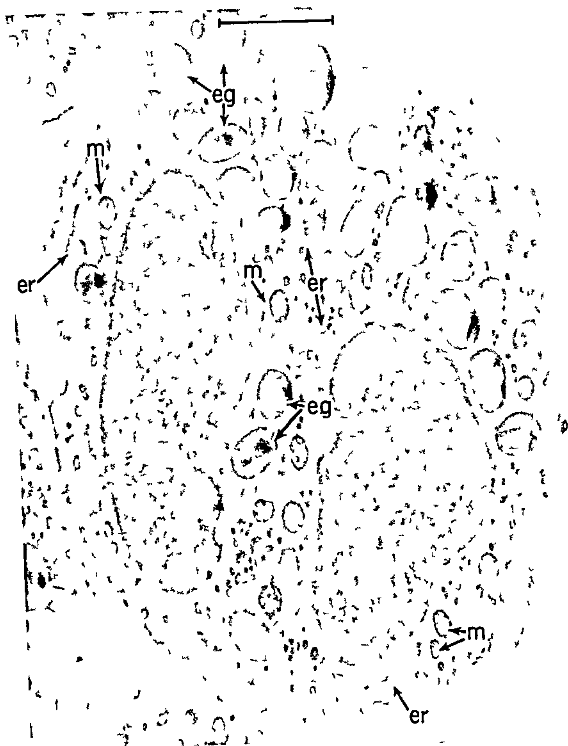
nd
m
ng
c
gp

1600 X

Eosinophil with bilobed nucleus A similar nucleus is represented by two separate lobes in the thin section of the eosinophil in the electron micrograph on the facing page Compare the granules in this light micrograph to those in the electron micrograph

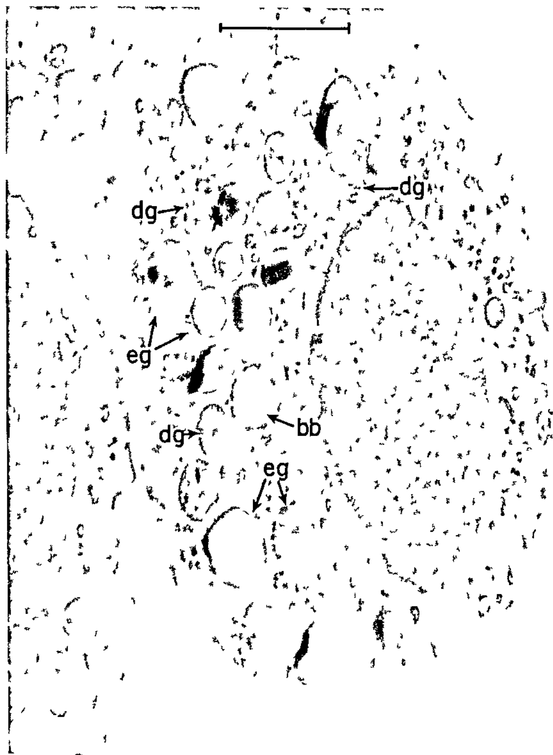
(Case 1f) 29 000 X

Eosinophil The specific granules *(eg)* show their dark inclusions to good advantage No basophilic bodies are present in this cell In eosino phils there is usually a random distribution of profiles of endoplasmic reticulum which are usually circular or oval in outline *(er)* but may be elongate Mitochondria normally present in mature eosinophils are indicated at *m* The density pattern in the two nuclear lobes is typical


eg
m
er
m
er
eg
m
er

(Case 1f) 33 000 X

Eosinophil The specific granules are conspicuous (*eg*) The main body of the granule is of medium density and contains a darker inclusion the shape of which varies considerably This particular section shows one typical basophilic body (*bb*) which consists of a homogeneous matrix surrounded by a dark margin These bodies are roughly circular in section but usually have an irregularly deckled outline Another type of structure commonly seen in eosinophils is characterized by finely granular or irregularly broken contents (*dg*) Detail of these granular types is presented on pages 61 and 63


dg
dg
eg
dg
bb
eg

(Case 1f) 33 000 X

Eosinophil The specific granules are conspicuous (*eg*) The main body of the granule is of medium density and contains a darker inclusion the shape of which varies considerably This particular section shows one typical basophilic body (*bb*) which consists of a homogeneous matrix surrounded by a dark margin These bodies are roughly circular in sec tion but usually have an irregularly deckled outline Another type of structure commonly seen in eosinophils is characterized by finely gran ular or irregularly broken contents (*dg*) Detail of these granular types is presented on *pages 61 and 63*


eg
bb
er
bb
eg

(Case 1f) 29 000 X

Typical eosinophil The specific granules are conspicuous (*eg*) and two basophilic bodies are present (*bb*) The two nuclear lobes show a characteristic density pattern The darker nucleoplasm is chiefly subjacent to the nuclear membrane with the lighter component more centrally located This pattern is very similar to the one characteristic of the nuclei of neutrophils There is considerably more endoplasmic reticulum (*er*) in the undifferentiated cytoplasm than in neutrophils


nu
L
go
eg
bb
er
m
eg
E
er

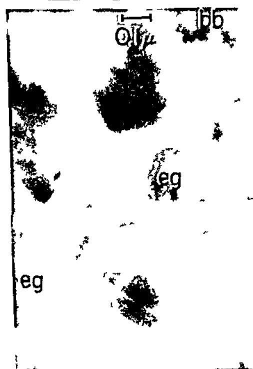
(Case 1f) 18 000 X

Lymphocyte (L) and eosinophil (E) The lymphocyte is typical with large mitochondria and Golgi zone (*go*) The pattern of nuclear density is characteristic and a nucleolus (*nu*) is present

The granules (*eg*) of the eosinophil are typical A basophilic body (*bb*) is present apparently cut nearly tangentially There are two mitochondria (*m*) and a moderate amount of endoplasmic reticulum (*er*) The density pattern of the nucleoplasm is characteristic


0 1μ
eg2
eg1
dg


0 1μ
eg
bb


0 1μ
bb
eg
eg


0 1μ
n
eg

Eosinophilic granules

Upper left (Case 11) 76 000 X

The granule at *eg1* is cut transversely with the dense component appearing as a rod extending nearly to the margins of the granule At *eg2* the granule is cut through its greatest diameter as is the dense component which is roughly circular The remaining three granules are cut in varying degrees of bias The evidence indicates that the dense component is a disk of fairly uniform thickness which may occupy the greatest width of the granule The lighter component shows an irregularly granular appearance at this level of magnification At *dg* there is an inclusion with irregular internal density

Upper right (Case 1f) 73 000 X

The eosinophilic granule at *eg* has its dark component represented by a number of haphazardly arranged crystalloids At *bb* there is a basophilic body

Lower left (Case 11) 72 000 X

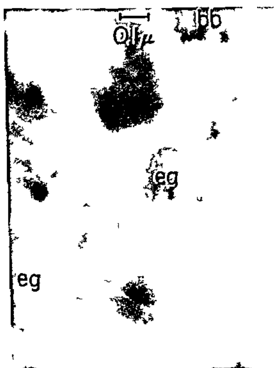
The granule in the lower part of the field has its dense component represented as three separate rods The two smaller structures (*eg*) are eosinophilic granules cut without involvement of the dense component At *bb* there is a portion of a basophilic body cut nearly tangentially

Lower right (Case 13) 80 000 X

The large granule (*eg*) in this field has its dense component present as both a large rod nearly vertically aligned and several smaller crystalloids The other granules are tangentially sectioned A small portion of the nucleus (*n*) is present with its double membrane


0.1μ
eg2
eg1
dg


0.1μ
eg
bb


0.1μ
bb
eg
eg


0.1μ
n
eg

Detail in granular cytoplasm of eosinophils

Upper left (Case If) 38 000 X

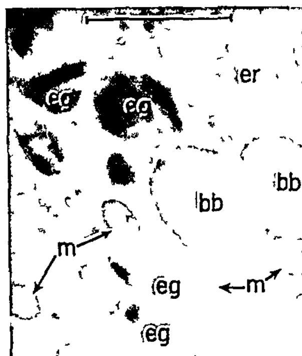
Basophilic bodies (*bb*) have a homogeneous matrix and a dense margin which tends to be irregular Eosinophilic granules are at *eg* There are four mitochondria present (*m*) and some endoplasmic reticulum (*er*)

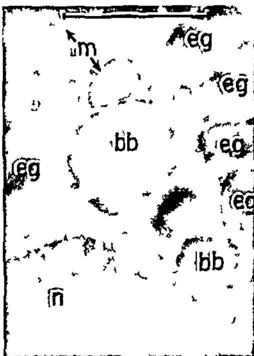
Upper right (Case If) 38 000 X

Two basophilic bodies (*bb*) are present Eosinophilic granules are evident (*eg*) and there are two mitochondria (*m*) A portion of the nucleus (*n*) is also visible

Lower (Case Ib) 47 000 X

These eosinophilic granules present some unusual features The granule at *eg*1 has dark components arranged transversely to its greatest diameter which is unusually large measuring 1 9 μ In *eg*2 there appears to be a granular content independent of the dark component The complex structure in *eg*3 is unidentified but is true ultrastructure within the granule Nuclear lobes (*n*) are indicated





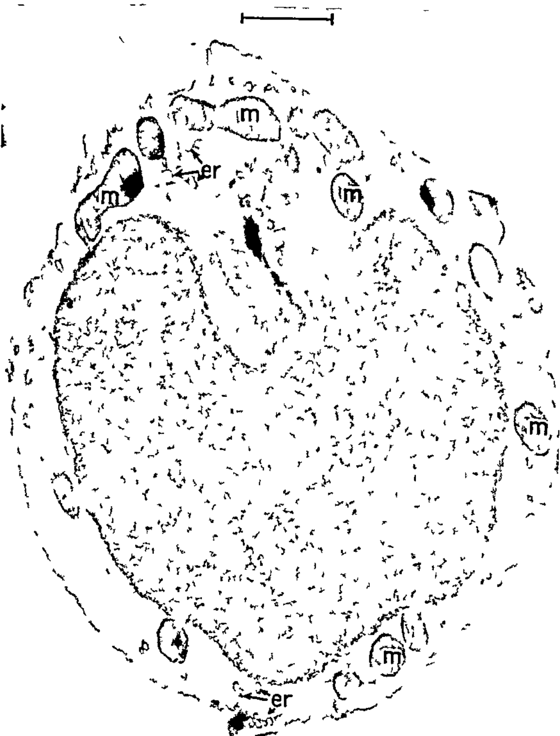
n
eg3
eg1
eg2
n
n

1600 X

Lymphocyte with typical structural features The relationship of its large nucleus to the sparse cytoplasm may be compared to the electron micrograph on the facing page Note that the nucleoplasm in the electron micrograph is of much smoother density

(Case 1a) 24 000 X

Lymphocyte The mitochondria (*m*) are large the oval ones measuring about 0 2 to 0 3 μ by 0 6 to 0 7 μ The rod shaped ones are about 1 1 to 1 25 μ long There is some endoplasmic reticulum (*er*) in the cytoplasm but in lymphocytes in general it is sparse The undifferentiated cytoplasm is clear compared to the highly speckled cytoplasm of monocytes The nucleoplasm has a fairly even density throughout Close examination reveals an approach to the two distinct densities characteristic of the nucleoplasm of neutrophils eosinophils and monocytes But wherever this occurs (in lymphocytes in general) its localization is haphazard and does not conform to any constant pattern as does the nucleoplasm of the above mentioned cells

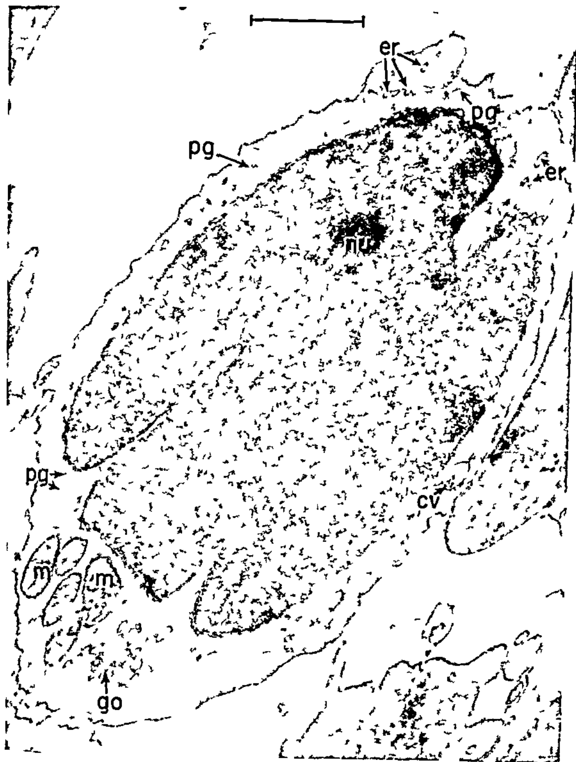

m
m
er
m
m
m
er

1600 X

Lymphocyte with typical structural features The relationship of its large nucleus to the sparse cytoplasm may be compared to the electron micrograph on the facing page Note that the nucleoplasm in the electron micrograph is of much smoother density

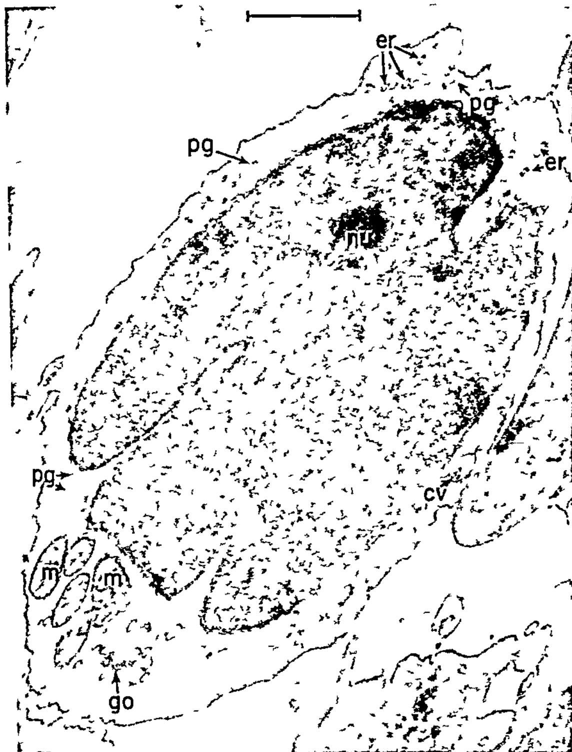
(Case Ia) 24 000 X

Lymphocyte The mitochondria (*m*) are large the oval ones measuring about 0 2 to 0 3 μ by 0 6 to 0 7 μ The rod shaped ones are about *1 1 to 1 25 μ long There is some endoplasmic reticulum (er) in the* cytoplasm but in lymphocytes in general it is sparse The undifferentiated cytoplasm is clear compared to the highly speckled cytoplasm of monocytes The nucleoplasm has a fairly even density throughout Close examination reveals an approach to the two distinct densities characteristic of the nucleoplasm of neutrophils eosinophils and monocytes But wherever this occurs (in lymphocytes in general) its localization is haphazard *and does not conform to any constant pattern as does the* nucleoplasm of the above mentioned cells


er
pg
pg
er
nu
pg
cv
m
m
go

(Case Ia) 29 000 X

Lymphocyte The mitochondria (*m*) visible in the section are lo cated at one pole of the cell near to the Golgi zone (*go*) There is a small amount of endoplasmic reticulum (*er*) and scattered Palade granules (*pg*) are present The nucleoplasm is of fairly even density except for the nucleolus (*nu*) A compound vacuole (*cv*) in the cyto plasm is described in detail elsewhere (page 116)


er
pg
pg
er
nu
pg
cv
m
m
go

(Case Ia) 28 000 X

Lymphocyte The mitochondria (*m*) are somewhat more numerous than usual but are of normal size (see page 65) Elsewhere in the cytoplasm are profiles of endoplasmic reticulum (*er*) scattered groups of Palade granules (*pg*) a large compound vacuole (*cv*) and three unidentified granules (*g*) which are described in greater detail elsewhere (page 120) The nucleus possesses considerable variation in density but this is typically patternless A nucleolus (*nu*) is visible and doubling of the nuclear membrane (*nm*) is also evident


m
m
m
m
cv
g
g
nm
pg
nm
pg
er
er
nu

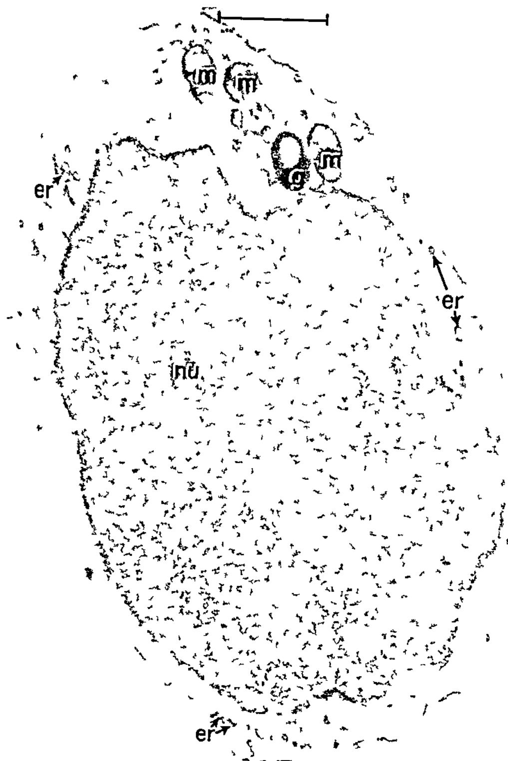
(Case Ia) 29 000 X

Lymphocyte The mitochondria (*m*) are of typical size and shape The circular and oval ones measure from 0 2 to 0 45 μ in diameter and the rod shaped one is about 1 2 μ long Most of the endoplasmic reticulum (*er*) is located at the opposite end of the cell There is some indication of two separate densities in the nucleoplasm but a lack of patternization is evident


m
m
m
er

(Case Ia) 29 000 X

Lymphocyte The mitochondria (*m*) conform to the measurements typical of th se cells There are the usual sparse profiles of endoplasmic reticulum (*er*) scattered throughout the cytoplasm The unusual granule (*g*) represents a type only occasionally seen and is considered in more detail elsewhere (page 122) The nucleoplasm is in general of even density but an approach to the two densities characteristic of other cells can be detected However these slight density differences are without a coherent pattern A nucleolus is present (*nu*) but it is quite diffuse and contrasts with the surrounding nucleoplasm somewhat less than usual


m
m
g
m
er
er
nu
er

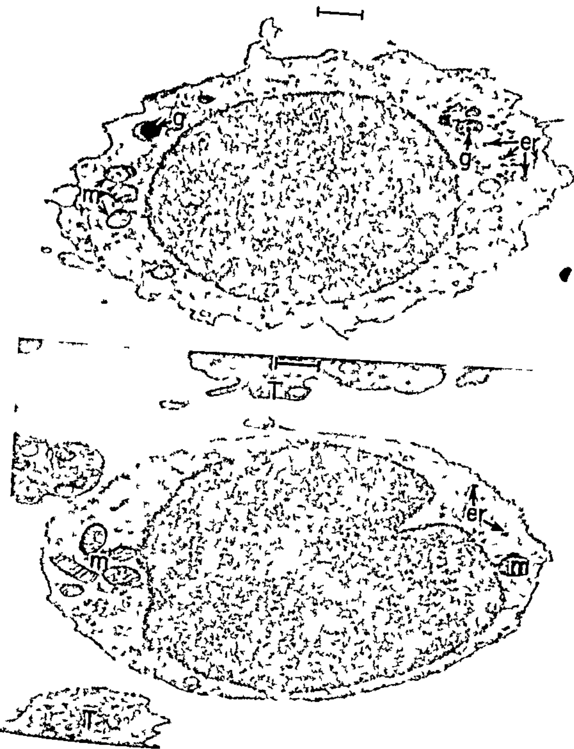
Lymphocytes Mitochondria (*m*) and endoplasmic reticulum (*er*) are present in both micrographs

Upper (Case 1a) 12 000 X

In the cytoplasm there are granules (*g*) which are not infrequent in lymphocytes and are described in more detail elsewhere (page 123) The nucleoplasm shows a uniform general density throughout The irregular contour is not unusual in these preparations but in this cell contrasts with the unusually symmetrical shape of the nucleus

Lower (Case 1a) 12 000 X

The nucleus shows some tendency to variations in density but this is characteristically patternless There are portions of blood platelets (*T*) in the field


g
m
g
er
T
er
m
im
T

Lymphocytes Mitochondria (*m*) and endoplasmic reticulum (*er*) are present in both micrographs

Upper (Case Ia) 12 000 X

In the cytoplasm there are granules (*g*) which are not infrequent in lymphocytes and are described in more detail elsewhere (page 123) The nucleoplasm shows a uniform general density throughout The irregular contour is not unusual in these preparations but in this cell contrasts with the unusually symmetrical shape of the nucleus

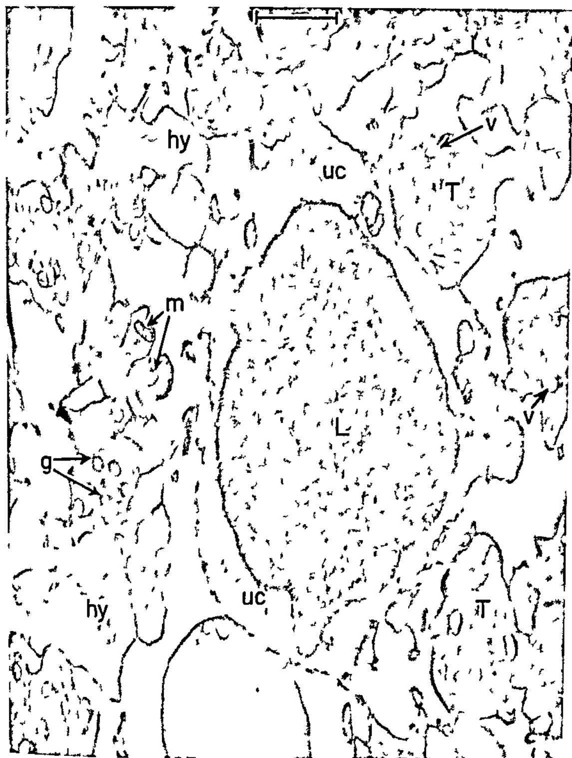
Lower (Case Ia) 12 000 X

The nucleus shows some tendency to variations in density but this is characteristically patternless There are portions of blood platelets (*T*) in the field


hy
uc
v
T
m
L
v
g
uc
hy
T

(Case 1b) 21 000 X

Lymphocyte (L) and blood platelets (T) Attention is called to the close resemblance between the appearance of the undifferentiated cytoplasm (*uc*) of the lymphocyte and the hyalomere (*h*) of the platelets The platelets are of typical appearance with granules (*g*) vacuoles (*v*) and mitochondria (*m*) Platelets are described in more detail on pages 88 to 101


hy
uc
v
T
m
L
v
g
hy
uc
T

1600 X

Monocyte showing relationship between nucleus and abundant cytoplasm This situation is well matched in the electron micrograph on the facing page However the nucleoplasmic densities differ somewhat and may be compared with those of the lymphocyte on page 64

(Case 1f) 27 000 X

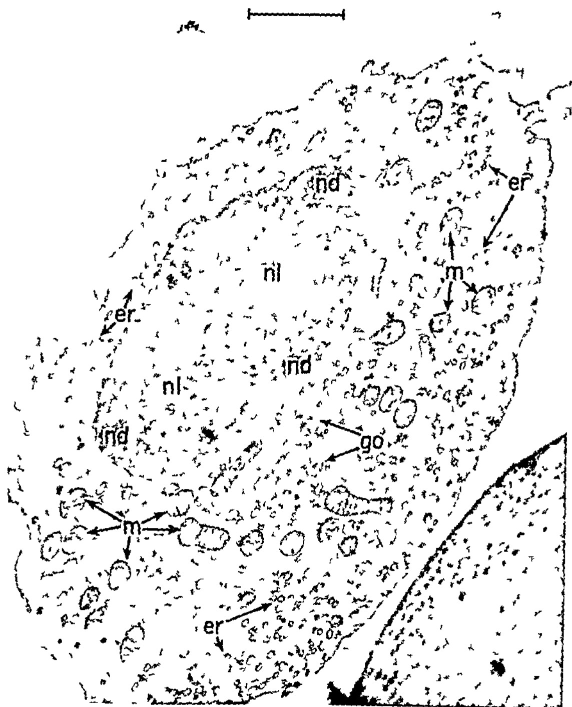
Monocyte The mitochondria (*m*) are small the circular and oval ones having diameters ranging from 0 15 to 0 4 μ with the average falling at about 0 2 μ (as compared to circular and oval mitochondria in lymphocytes which vary from 0 2 to 0 7 μ with an average at about 0 45 μ) There are abundant profiles of endoplasmic reticulum (*er*) distributed throughout the cytoplasm The Golgi zone (*go*) is visible The cytoplasm in general is dark and has a speckled appearance as compared with the cytoplasm of the lymphocyte which is relatively clear The nucleoplasm varies in density and is patternized The denser nucleoplasm forms a definite although irregular band (*nd*) subjacent to the nuclear membrane and is haphazardly mixed with the lighter nucleoplasm (*nl*) in the central part of the nucleus


er
m
nd
go
er
er
nl
m
er

MONOCYTES

(Case 4) **26 000 X**

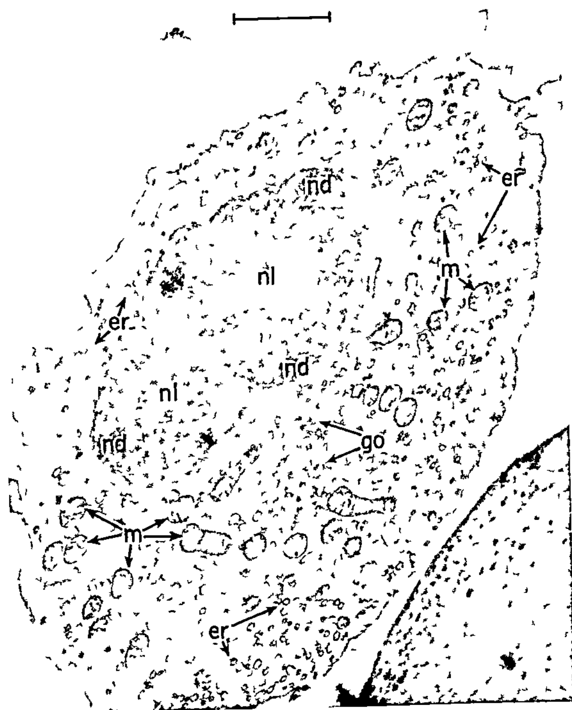
Monocyte The mitochondria (*m*) are typically small the circular and oval ones measuring 0 15 to 0 4 μ The rod shaped ones do not exceed 0 8 μ in length The profiles of endoplasmic reticulum (*er*) are typically abundant They contribute much to the generally speckled appearance of the cytoplasm as compared with that of the lymphocyte in which the endoplasmic reticulum is scant The Golgi zone (*go*) is visible The nucleoplasm has two distinct densities with the denser portion (*nd*) near the nuclear membrane and the lighter component (*nl*) more centrally located


er
nd
er
m
nl
er
nd
nl
nd
go
m
er

MONOCYTES

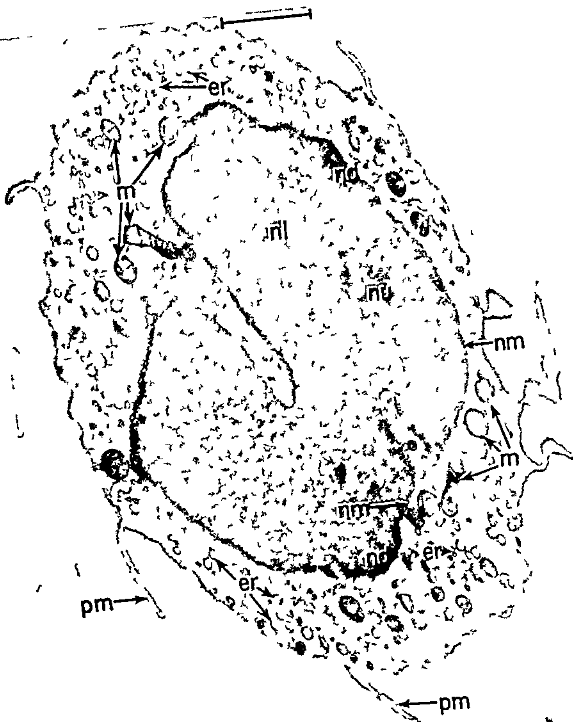
(Case 4) 26 000 X

Monocyte The mitochondria (m) are typically small the circular and oval ones measuring 0.15 to 0.4 μ The rod shaped ones do not exceed 0.8 μ in length The profiles of endoplasmic reticulum (er) are typically abundant They contribute much to the generally speckled appearance of the cytoplasm as compared with that of the lymphocyte in which the endoplasmic reticulum is scant The Golgi zone (go) is visible The nucleoplasm has two distinct densities with the denser portion (nd) near the nuclear membrane and the lighter component (nl) more centrally located


nd
er
m
nl
er
nd
nl
go
nd
m
er

(Case Ia) 24 000 X

Monocyte with typical structural detail The mitochondria (*m*) are of normal size for a monocyte (pages 79 and 81) and the rod shaped ones do not exceed 0 75 μ in length There are many profiles of endo plasmic reticulum (*er*) and the over all appearance of the cytoplasm is characteristically speckled The nucleoplasm tends to have its denser component (*nd*) along the nuclear membrane with a lighter central area (*nl*) A nucleolus (*nu*) is present In many areas the nuclear membrane (*nm*) is clearly double The fine external extensions of plasma membrane (*pm*) are probably analogous to those described elsewhere (pages 20 and 48)


er
m
nd
nl
nu
nm
m
nm
nd
er
er
pm
pm

MONOCYTES

(Case 1f) 24 000 X

Monocyte Although there are fewer mitochondria (*m*) than usual, *they are typically small Profiles of endoplasmic reticulum* (*er*) are common The nucleoplasm shows its denser component (*nd*) very clearly and it characteristically forms a dark band subjacent to the nuclear membrane A portion of a neutrophil (*N*) is at the left In its nucleus the similar but more evident pattern of nuclear densities may be compared with those of the monocyte

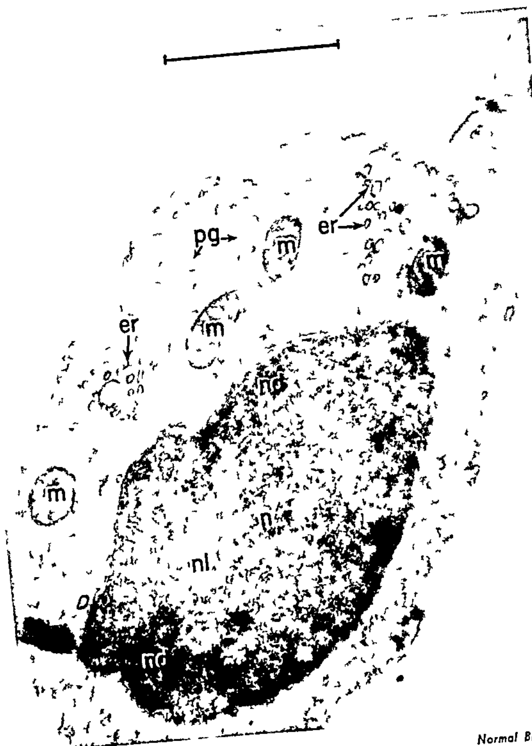

m
er
ind
ind
er
ind
N
er
m
ind
er
er

(Case 4) 46 000 X

Unidentified agranulocyte This cell was purposely chosen to illustrate the ambiguity that may arise in trying to distinguish monocytes from lymphocytes The criteria useful in distinguishing these two cell types from each other (see pages 64 to 77 for lymphocytes and pages 78 to 85 for monocytes) may be applied to this cell as follows

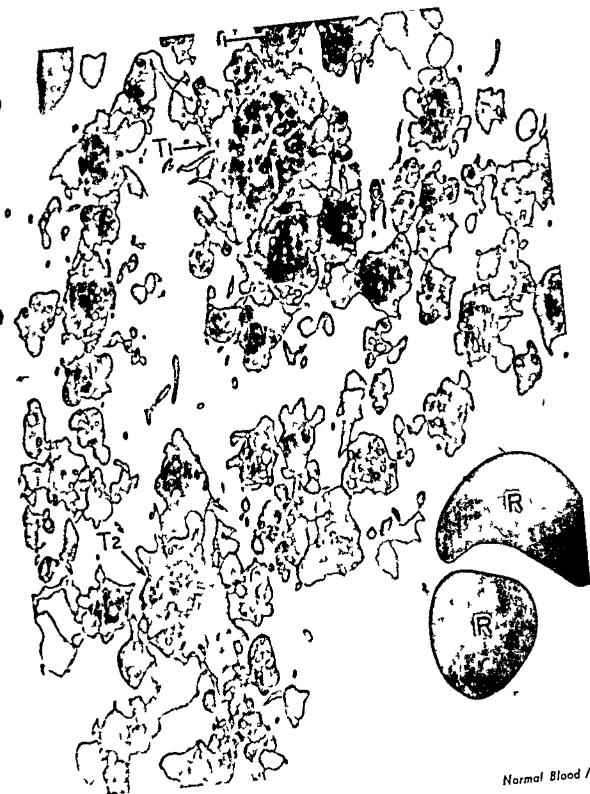
The mitochondria (*m*) measure from 0 2 to 0 5 μ but this size range might fit either cell type and they are not numerous enough to draw an average The endoplasmic reticulum (*er*) is somewhat more copious than in lymphocytes but is localized chiefly in two areas and is not randomly distributed as is usually the case in monocytes The undifferentiated cytoplasm has many Palade granules (*pg*) and is darker than in most lymphocytes but not as speckled as in typical monocytes The nucleus (*n*) shows two densities dark (*nd*) and light (*nl*) with a tendency for the dark nucleoplasm to be close to the nuclear mem brane But the pattern does not conform well to that usually found *in either lymphocytes or monocytes*

Occasionally agranulocytes such as this one are encountered and positive identification is not possible But most agranulocytes seen in these preparations can be identified as either lymphocytes or monocytes


pg
m
er
m
er
m
nd
m
n
nl
nd

[Case 1b] 12 000 X

Irregular outlines of platelets prepared by this technique Most of the platelets measure between 0 75 and 2 25 μ in diameter However con spicuously larger ones (T_1 T_2) are routinely observed in normal blood samples The two illustrated here measure 2 5 by 4 μ (T_1) and 2 by 3 μ (T_2) Two red blood cells are at *R*


T1
T2
R
R

(Case 1f) 22 000 X

Chromomere and hyalomere of blood platelets The darker chromomere (*ch*) usually centrally located and the lighter hyalomere (*hy*) which is peripheral conform to the pattern well recognized in light microscopy The granules (*g*) of the chromomere are dark and of fairly uniform size their diameters ranging from 0 15 to 0 3 μ Note that the undifferentiated protoplasm of the chromomere is darker than that of the hyalomere The plasma membrane (*pm*) of platelets is usually well defined and quite dense


hy
pm
pm
g
hy
ch
g

(Case Ib) 31 000 X

Granules and vacuoles of blood platelets The granules (*g*) in the chromomeres present no unusual features There are numerous vacuoles (*v*) in the protoplasm and these are a conspicuous feature of platelets prepared by this technique They probably correspond to the contractile vacuoles demonstrated in light microscope studies Although mitochondria are present in blood platelets they only rarely show their internal structure to good advantage (see page 99) Their usual appearance in these preparations is illustrated at *m* They are often of the same size and shape as the granules


g
v
v
m
v
g
v

(Case 1b) 23 000 X

Dense plasma membrane (*pm*) *of platelets* This is a characteristic feature of platelets prepared by this technique The granules (*g*) of the chromomeres (*ch*) and the relatively structureless hyalomere (*hy*) are readily identified Note in this and the preceding and following illustrations of platelets that the usual concept of a centrally located chromomere surrounded by a peripherally located hyalomere is not always borne out The chromomere often extends to the surface (*ch*1) and sometimes occupies practically the entire section of the platelet (*ch*2)


ch 2
ch 1
hy
g
pm
g
ch 2
hy
pm

(Case Ib) 23 000 X

Dense plasma membrane (pm) of platelets This is a characteristic feature of platelets prepared by this technique The granules (*g*) of the chromomeres (*ch*) and the relatively structureless hyalomere (*hy*) are readily identified Note in this and the preceding and following illustrations of platelets that the usual concept of a centrally located chromomere surrounded by a peripherally located hyalomere is not always borne out The chromomere often extends to the surface (*ch*1) and sometimes occupies practically the entire section of the platelet (*ch*2)


ch
g
hy
hy
hy
hy
hy
g
ch

(Case 1b) 23 000 X

Hyalomere of blood platelets There is somewhat more of the light hyalomere (*h*) present than in most fields Wherever the chromomere (*ch*) and its constituents are present it does not have much greater density in this particular micrograph than the hyalomere This gives the entire field a narrower range of densities than usual The granules (*g*) where present are quite light This low-contrast appearance is not unusual


m
m
g
v
g
m
m
m
v
m
m

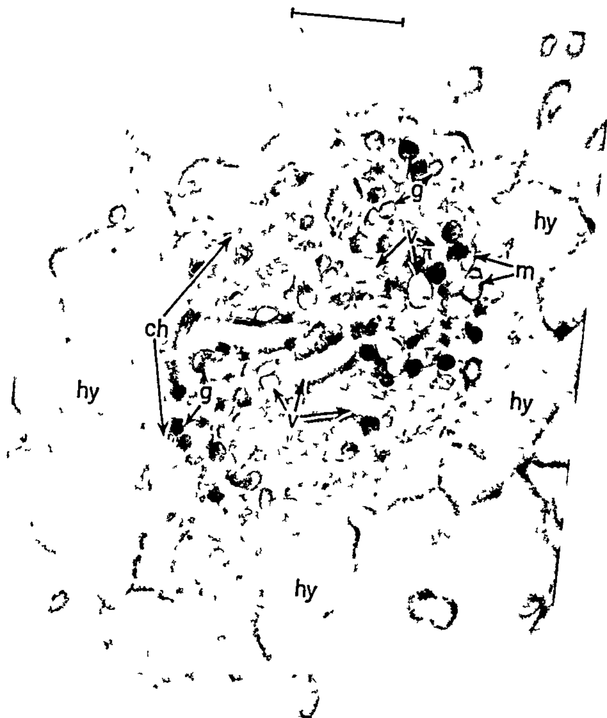
(Case Ib) 23 000 X

Mitochondria of blood platelets An unusually large number of mito chondria (*m*) are present As is characteristic of mitochondria in plate lets these have poorly defined structural features There are a few granules (*g*) most of which have a low density that hardly contrasts with the surrounding matrix of the chromomere Some contractile vacuoles (*v*) are present


m
g
m
m
v
g
m
v
m
m

(Case 1f) 30 000 X

Giant platelet This platelet is somewhat larger than usual measuring about 5 by 6 μ in over all diameter The chromomere (*ch*) largely filled with dark granules (*g*) forms a well circumscribed oval about 2 7 by 3 3 μ The dense granules are very variable in their size measuring up to 0 25 μ but much of this may be due to the fact that the section seldom passes through their exact center There are numerous vacuoles (*v*) in both the chromomere and the hyalomere (*hy*) Some recognizable mitochondria (*m*) are present but these in platelets are seldom so clearly defined as in living cells


g
hy
v
m
ch
hy
g
v
hy
hy

Mitochondria of leukocytes The fields here presented are from lym phocytes

Upper (Case Ia) 64 000 X

The double membrane organization of mitochondria (*m*) is evident in this micrograph Both the external membrane (*em*) and the cristae mitochondriales (*cm*) are visualized as two dark lines bordering a clear interspace The undifferentiated matrix of the mitochondria varies from light to moderately dense and contains occasional dense granules (see lower left below) A portion of a nucleus (*n*) is present and Palade granules (*pg*) are randomly scattered throughout the cytoplasm

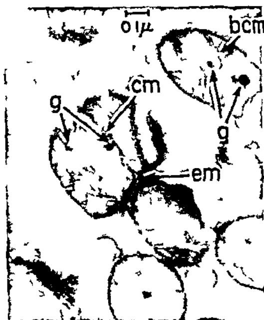
Lower left (Case If) 66 000 X

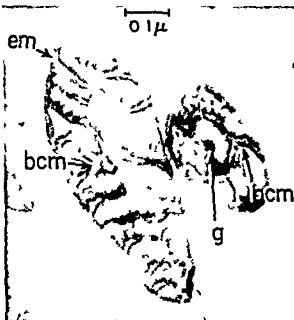
The cristae mitochondriales (cm) are derived as infoldings of the inner member of the external membrane (*em*) Dense granules (*g*) are often seen in the matrix of the mitochondria Branchings of the cristae are often observed usually in the form of a Y (*bcm*)

Lower right (Case Ia) 120 000 X

A branched crista (bcm) is illustrated in the mitochondrion on the right In the mitochondrion on the left there is an unusually complex branching of a crista in which four (or five) components may be counted At *g* there is a granule in the matrix of the usual size and density Elsewhere in the matrix of this mitochondrion there are smaller dense granules At *em* a crista is derived from the internal member of the external double membrane

cm
em
pg
m
em
pg
cm
n


0.1 μ
bcm
cm
g
g
em


0.1 μ
em
bcm
bcm
g

Mitochondrial structure

Upper (Case Ia) 120 000 X

The characteristic doubling of both the external membrane (*em*) and the cristae mitochondriales (*cm*) is visible There are a few dense granules (*g*) in the mitochondrial matrix which elsewhere has a faintly speckled appearance as if containing smaller granules of very slightly greater density than the matrix itself Palade granules (*pg*) are randomly scattered throughout the cytoplasm Part of a nucleus (*n*) is present

Lower (Case Ia) 87 000 X

Certain of the internal relationships of the cristae mitochondriales are fortuitously illustrated in these mitochondria At cm_1 two cristae can be observed to arise from the external membrane each on a different side of the mitochondrion and to extend some distance but not all the way across its width toward the opposite side At cm_2 three cristae are apparently isolated from the external membrane but this is because their attachment is out of the plane of section However the derivation of the cristae from the external membrane must occur along a broad base since they frequently traverse the entire diameter of the mitochondrion with attachments at both sides (cm_3) Nevertheless the cristae mitochondriales constitute incomplete partitions across the width of the mitochondrion Palade granules (*pg*) are conspicuous

0.1 μ

cm

g

em

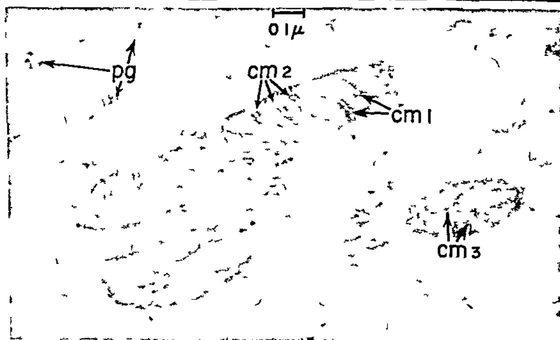
g

cm

g

pg

n



(Case 1a) 280 000 X

Mitochondrion Both the external membrane (*em*) and the cristae mitochondriales (*cm*) are double membranes consisting of two dark lines separated by a light interspace The cristae are derived as infoldings of the inner member of the external membrane (*cmd*) The cristae sometimes branch (*bcm*) and are incomplete partitions across the width of the mitochondrion Dense granules (*g*) may occur in the matrix Each dense member of the double membrane forming a crista is known to be double in itself (*dc*) forming a secondary double membrane system

A portion of the nucleus (*n*) with its double nuclear membrane (*nm*) is present Note the Palade granules (*pg*) scattered randomly throughout the cytoplasm

The cell containing this mitochondrion a lymphocyte is illustrated in its entirety on page 69 and in part on page 121

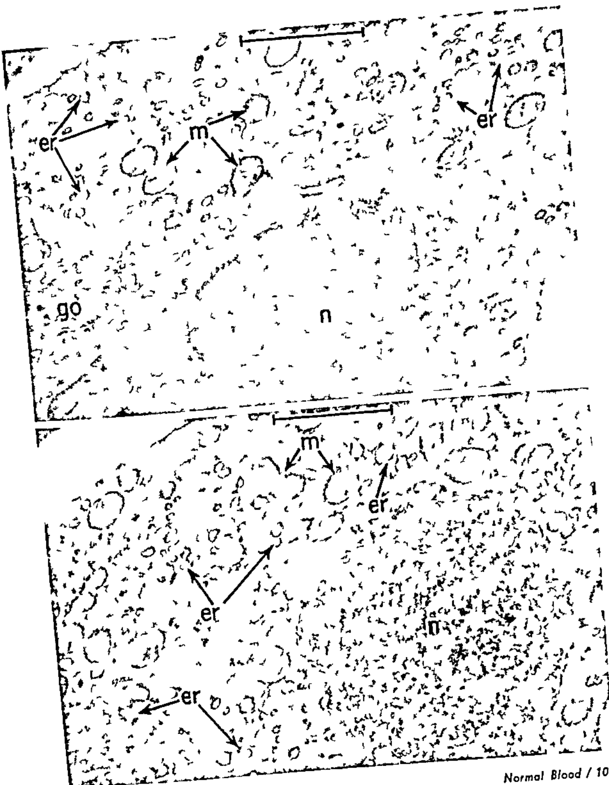

01 μ
pg
dc
g
em
cmd
bcm
cm
g
cm
cmd
n
nm

(Case 1a) 280 000 X

Mitochondrion Both the external membrane (*em*) and the cristae mito chondriales (*cm*) are double membranes consisting of two dark lines separated by a light interspace The cristae are derived as infoldings of the inner member of the external membrane (*cmd*) The cristae sometimes branch (*bcm*) and are incomplete partitions across the width of the mitochondrion Dense granules (*g*) may occur in the matrix Each dense member of the double membrane forming a crista is known to be double in itself (*dc*) forming a secondary double membrane system

A portion of the nucleus (*n*) with its double nuclear membrane (*nm*) is present Note the Palade granules (*pg*) scattered randomly throughout the cytoplasm

The cell containing this mitochondrion a lymphocyte is illustrated in its entirety on page 69 and in part on page 121



er
m
er
go
n
m
er
er
n
er

Endoplasmic reticulum These fields both detail of monocytes were chosen to illustrate the small round or oval profiles of endoplasmic reticulum that are characteristic of normal mature leukocytes in general Cisternal forms are practically never seen except in the very rare plasma cells

Upper (Case 4) 33 000 X

The small round or oval profil s of endoplasmic reticulum (*er*) thickly populate the cytoplasm Note the Golgi zone (*go*) which bears close resemblance to endoplasmic reticulum It can be distinguished by the flattened sacs and the total concentration of its vacuoles Mitochondria (*m*) and a portion of the nucleus (*n*) are present This cell is illus trated in its entirety on page 81

Lower (Case 10) *31 000 X*

The endoplasmic reticulum (*er*) in this field is characteristic Note that some of the profiles are distinctly larger than others Mitochondria (*m*) and a portion of the nucleus (*n*) are present

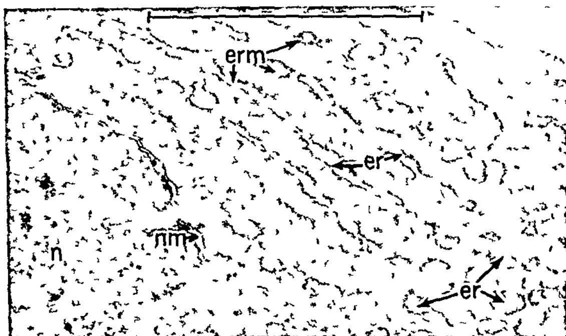
Endoplasmic reticulum from plasma cells The upper one was found in normal peripheral blood and the lower one occurred in a person with eosinophilia

Upper (Case Ia) *72 000 X*

The endoplasmic reticulum (*er*) is present in the form of both profiles of flattened sacs (cisternae) and circular or oval profiles of vacuolar appearance A dense membrane (*erm*) limits the reticulum from the surrounding cytoplasm which is somewhat more dense than the interior of the reticulum itself A portion of a nucleus (*n*) with its characteristic double membrane (*nm*) is present

Lower (Case II) *110 000 X*

The endoplasmic reticulum (*er*) is visible in the form of flattened sacs (cisternae) which occupy most of the field and as circular and oval profiles to the left The limiting membrane (*erm*) is dense and is clearly visible throughout most of its extent The cytoplasm has numerous *Palade granules (pg) scattered randomly through it The interior of* the reticulum is less dense than the cytoplasm and this seems chiefly due to the fact that there are no Palade granules or other organized structures present in the reticulum


erm
er
nm
n
er

0.1 μ
pg
er
er
er
er
er
er
erm
er
pg
erm
er
er

Palade granules These micrographs were chosen to illustrate the small particulate component of the cytoplasm known to be rich in ribo nucleic acid These dense spherules are usually referred to as *Palade granules*

Upper (Case 4) 34 000 X

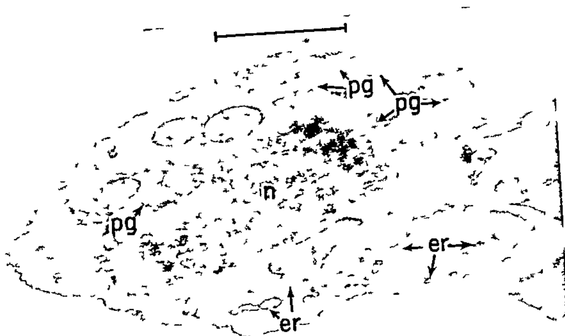
The dense Palade granules (*pg*) are randomly distributed throughout the cytoplasm of this agranulocyte In blood leukocytes these granules do not show affinity for any particular structure as they do for the endoplasmic reticulum in some cells elsewhere in the body The endo plasmic reticulum (*er*) in this cell is not associated with the Palade granules

Lower left (Case 4) 58 000 X

Note that the Palade granules cannot be morphologically distinguished from the granules of the nucleus (*n*)

Lower right (Case 1a) 65 000 X

This field is fairly evenly stippled with Palade granules (*pg*) They range from 100 to 150 A in diameter and are always dense enough to be clearly distinguished from the undifferentiated cytoplasm Note that they do not show any selective affinity for either the endoplasmic reticulum (*er*) or the mitochondria (*m*)



0·1μ

pg
n
pg

pg
er
pg
m
m

Palade granules These micrographs were chosen to illustrate the small *particulate component of the cytoplasm known to be rich in ribo* nucleic acid These dense spherules are usually referred to as *Palade granules*

Upper (Case 4) 34 000 X

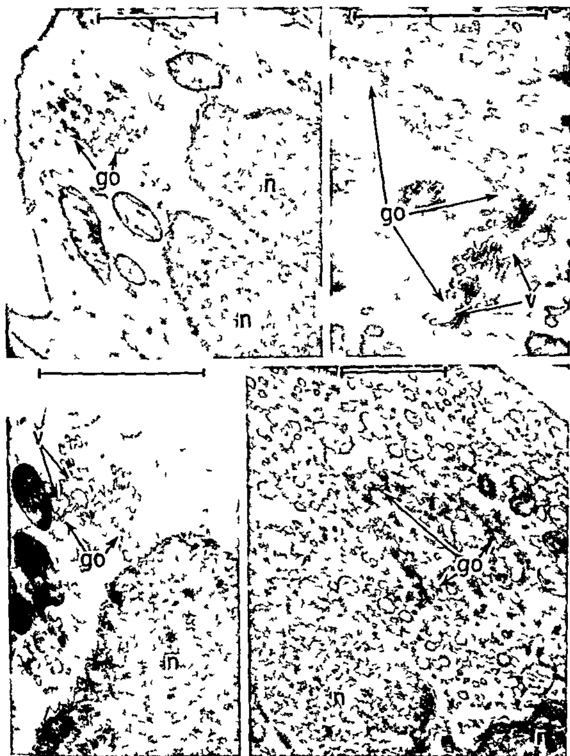
The dense Palade granules (*pg*) are randomly distributed throughout the cytoplasm of this agranulocyte In blood leukocytes these granules do not show affinity for any particular structure as they do for the endoplasmic reticulum in some cells elsewhere in the body The endo plasmic reticulum (*er*) in this cell is not associated with the Palade granules

Lower left (Case 4) 58 000 X

Note that the Palade granules cannot be morphologically distinguished from the granules of the nucleus (*n*)

Lower right (Case 1a) 65 000 X

This field is fairly evenly stippled with Palade granules (*pg*) They *range from 100 to 150 A in diameter and are always dense enough* to be clearly distinguished from the undifferentiated cytoplasm Note that they do not show any selective affinity for either the endoplasmic reticulum (*er*) or the mitochondria (*m*)


go
n
n
go
v
v
go
n
go
n
n

Golgi zone

Upper left (Case Ia) 31 000 X

The appearance of the Golgi zone (*go*) in this field is characteristic It consists of flattened sacs which have the appearance of double mem branes and numerous profiles of small vacuolar structures which are round or circular Its extent is usually fairly well delimited from the surrounding cytoplasm Two nuclear lobes (*n*) are present

Upper right (Case Ia) 50 000 X

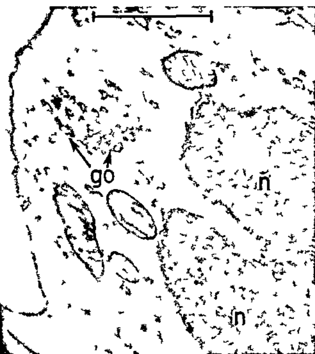
In this micrograph the Golgi zone (*go*) is in the form of a bent line extending nearly the entire length of the field The flattened sacs and circular or oval vacuoles are clearly visible Certain larger vacuoles (*v*) appear to be ones which have not flattened out and thus contain a clear apparently empty area The cell is a neutrophil

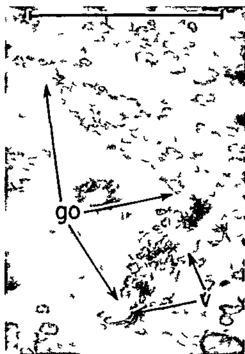
Lower left (Case Ia) 44 000 X

The Golgi zone here presents both flattened sacs and oval and round vacuoles There are larger vacuoles (*v*) which apparently differ only in size and shape from the remainder of them A portion of the nucleus (*n*) is present

Lower right (Case 10) 27 000 X

The Golgi zone in this cell contrasts less than usual with the surround ing cytoplasm This is because of the greater density of the cytoplasm which is due to the large number of profiles of endoplasmic reticulum Palade granules etc contained in it Portions of the nucleus (*n*) are visible The cell is a monocyte


go
n
n


go
v


v
go
n


go
n
n

Compound vacuoles These micrographs were chosen to illustrate certain vacuoles not demonstrable in light microscopy which contain smaller vacuoles within them

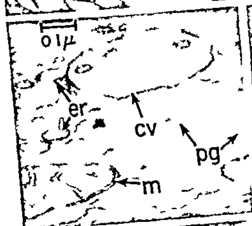
Upper (Case 1a) 25 000 X
This lymphocyte contains a compound vacuole (cv) located in the narrow band of perinuclear cytoplasm at one side of the cell Such vacuoles are apparently haphazardly placed in the cell without definite positional association Although they are most common in lymphocytes they may occur in any of the leukocytes

Middle left (Case 1a) *93 000 X*
The compound vacuole (*cv*) contains five or six smaller vacuoles which are haphazardly arranged within it Its membrane may be contrasted with that of the mitochondrion (*m*) near by Some Palade granules (*pg*) and profiles of endoplasmic reticulum (*er*) are evident

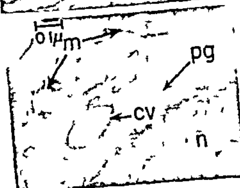
Middle right (Case 1f) *57 000 X*
Two compound vacuoles are illustrated here Their contained vacuoles are typical and for size and shape are indistinguishable from the small vacuoles (*v*) scattered throughout the cytoplasm A portion of a nucleus (*n*) is present

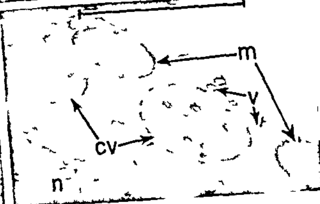
Lower left (Case 1a) 76 000 X
This compound vacuole contains smaller vacuoles whose density contrasts only slightly with the light surrounding matrix Portions of two mitochondria (*m*) are present and Palade granules (*pg*) are fairly evenly distributed throughout the cytoplasm A portion of the nucleus (*n*) is present

Lower right (Case 1f) 45 000 X
These two compound vacuoles have distinctly different appearances The one at the right contains small vacuoles which are indistinguishable from those in the surrounding cytoplasm (*v*) The vacuole at the left has much smaller inclusions than usual but they are definitely vacuolar The matrix of this compound vacuole is distinctly darker than that of the one on the right Two mitochondria (*m*) and a portion of the nucleus (*n*) are present


0 1 μ
er
cv
pg
m


v
cv
n


0 1 μ
m
pg
cv
n


m
v
cv
n

Compound vacuoles These micrographs were chosen to illustrate certain vacuoles not demonstrable in light microscopy, which contain smaller vacuoles within them

Upper (*Case Ia*) 25 000 X

This lymphocyte contains a compound vacuole (*cv*) located in the narrow band of perinuclear cytoplasm at one side of the cell Such vacuoles are apparently haphazardly placed in the cell without definite *positional association Although they are most common in lymphocytes* they may occur in any of the leukocytes

Middle left (Case Ia) 93 000 X

The compound vacuole (*cv*) contains five or six smaller vacuoles which *are haphazardly arranged within it Its membrane may be contrasted* with that of the mitochondrion (*m*) near by Some Palade granules (*pg*) and profiles of endoplasmic reticulum (*er*) are evident

Middle right (Case If) 57 000 X

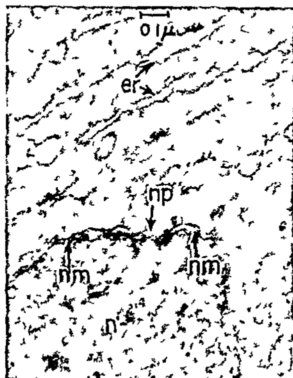
Two compound vacuoles are illustrated here Their contained vacuoles are typical and for size and shape are indistinguishable from the small vacuoles (*v*) scattered throughout the cytoplasm A portion of a nucleus (*n*) is present

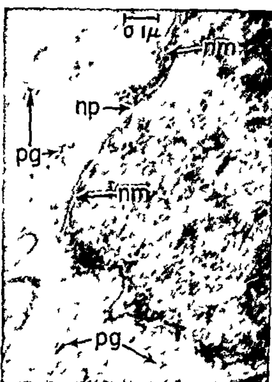
Lower left (Case Ia) 76 000 X

This compound vacuole contains smaller vacuoles whose density contrasts only slightly with the light surrounding matrix Portions of two mitochondria (*m*) are present and Palade granules (*pg*) are fairly evenly distributed throughout the cytoplasm A portion of the nucleus (*n*) is present

Lower right (Case If) 45 000 X

These two compound vacuoles have distinctly different appearances The one at the right contains small vacuoles which are indistinguishable *from those in the surrounding cytoplasm* (*v*) *The vacuole at the left* has much smaller inclusions than usual but they are definitely vacuolar The matrix of this compound vacuole is distinctly darker than that of the one on the right Two mitochondria (*m*) and a portion of the nucleus (*n*) are present


0 1μ
er
np
nm
nm
n


0 1μ
nm
np
pg
nm
pg

0 1μ
er
cv
pg
m
m
m
nm
np
nm

Nuclear pores The outer component of the nuclear membrane turns inward and joins the inner component to form one margin of the pore The other margin is similarly formed from the other direction

Upper left (Case Ia) 71 000 X

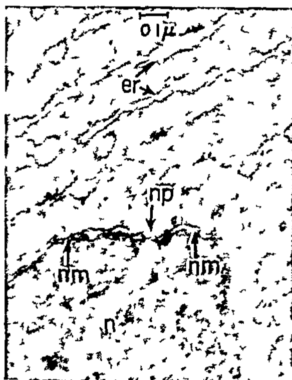
The nucleus (*n*) with its double nuclear membrane (*nm*) is clearly visible A nuclear pore (*np*) about 370 A in diameter is visible In this particular cell (a plasma cell from normal blood) the endoplasmic reticulum (*er*) is arranged in the form of parallel flattened sacs (cisternae)

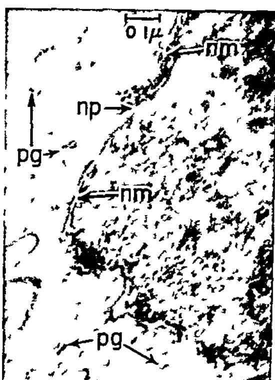
Upper right (Case Ia) 96 000 X

This micrograph illustrates a nuclear pore (*np*) in the clearly double nuclear membrane (*nm*) This pore measures about 480 A Palade granules (*pg*) are evident in the cytoplasm

Lower (Case Ia) 119 000 X

The double nuclear membrane (*nm*) is interrupted at *np* to form a nuclear pore which is 620 A wide In the cytoplasm are typical mito chondria (*m*) endoplasmic reticulum (*er*) Palade granules (*pg*) and part of a compound vacuole (*cv*)


0.1μ
er
np
nm
nm
n


0.1μ
nm
np
pg
nm
pg

0.1μ
er
cv
pg
m
m
m
nm
np
nm

General features of ultrastructure of protoplasm The cell is a lymphocyte illustrated in its entirety on page 69

Upper (Case Ia) 51 000 X

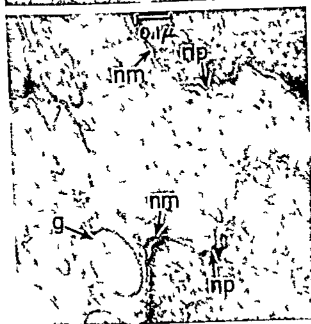
Two nuclear lobes (*n*) are conspicuous and the nuclear membrane (*nm*) shows a prominent doubling throughout most of its extent The mitochondria (*m*) are typical and show considerable variety in the cristae and granules in the matrix Palade granules (*pg*) and compound vacuoles (*cv*) are present There are three granules (*g*) in the cytoplasm which are not positively identified but which probably correspond to the azurophil granules well known in light microscopy They possess a dense outer membrane with a clear band just internal to it and a large homogeneous matrix of medium density

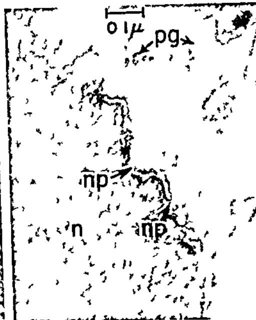
Lower left (Case Ia) 96 000 X

This micrograph illustrates detail from the field above at a higher magnification Two nuclear pores are present (*np*) in the clearly double nuclear membrane (*nm*) The nuclear membrane itself is of typical thickness measuring about 210 A over all with dense components somewhat less than one third of this thickness One of the unidentified granules (*g*) described above is visible It measures about 0 22 by 0 13 μ

Lower right (Case Ia) 96 000 X

Two nuclear pores (*np*) are visible in this field The granularity of the nucleoplasm (*n*) is typical These granules are densely packed but cannot morphologically be distinguished from the Palade granules (*pg*) of the cytoplasm which are always more sparsely distributed


0.1μ
nm
np
nm
g
np


0 1μ
pg
np
n
np

Odd granules The structure of these granules does not conform to that of any known organoid or inclusion and no identification is at present possible

Upper left (Case 1a) 39 000 X

The granule ($g1$) is part of a lymphocyte It is very dense and contains an oval lucid area Otherwise it appears to be essentially structureless It measures about 0 5 by 0 3 μ roughly the same size as the mitochondria (m) Below it ($g2$) are several unidentified granules about 0 1 to 0 2 μ in diameter

Upper right (Case 1a) 27 000 X

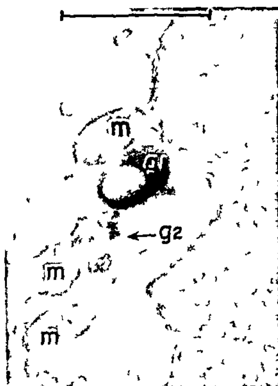
This granule (g) from a lymphocyte has a moderately dense body and contains an oval inclusion of high density It measures about 0 3 by 0 45 μ and is comparable in size to the mitochondria (m)

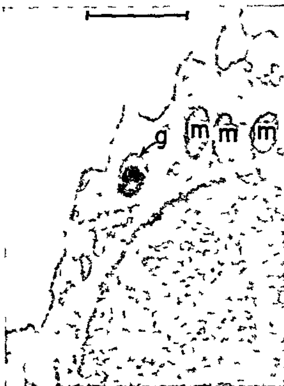
Lower left (Case 10) 28 000 X

These granules are not unlike the ones illustrated above but are in the cytoplasm of a monocyte There is considerable variation in size among the four granules present There is abundant endoplasmic reticulum in the surrounding cytoplasm

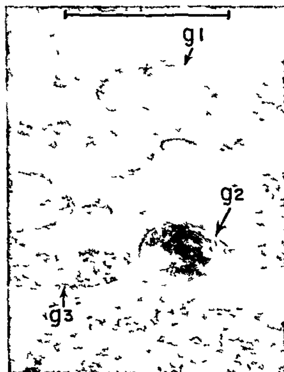
Lower right (Case 1a) 44 000 X

The granule at $g1$ is made up of dense closely packed laminae It measures about 0 45 by 0 7 μ with laminae about 75 A thick At $g2$ there is another granule apparently of the same kind The elongate structure at $g3$ is also unidentified The cell is a lymphocyte


m
g
g2
m
m


g
m
m
m


g
g


g1
g2
g3



GRANULOCYTIC LEUKEMIA

Cases of subacute and chronic granulocytic (subacute and chronic myelogenous) leukemia present a variety of cell types which are chiefly immature forms in the granulocytic series (neutrophilic eosinophilic basophilic) Developing forms of the erythrocytic series are also occasionally present In general the immature forms are found in the later stages of development The granulocytes are chiefly in the metamyelocyte or myelocyte stage promyelocytes and myeloblasts being only occasionally encountered

The terminology adopted for our descriptions is one frequently used in light microscopy A *promyelocyte* is understood to be the earliest form recognizable by its specific granules which are not yet numerous In the next stage the *myelocyte* the granules have reached their mature number In *metamyelocytes* the full population of granules of course persists but the nuclear shape has changed to a banded form Since nuclear shape is not a reliable criterion in thin sections and the number of granules present in the thin plane of the section may vary haphazardly other criteria must be used in electron microscopy The most valuable single criterion of developmental status in these preparations is nucleoplasmic differentiation In early forms (myeloblasts promyelocytes) the density of the nucleoplasm is even and usually light In older forms a denser nucleoplasm begins to form inside the nuclear membrane and becomes easily recognizable at the myelocyte stage The dark nucleoplasm later develops into an irregular but very distinct band inside the nuclear membrane and extends deep into the nucleus in irregular clumps At the metamyelocyte stage it has reached the mature pattern but the contrast between its light and dark components is often not so great as in mature cells

The endoplasmic reticulum is also a useful indicator of the developmental stage of a given cell In promyelocytes it is most copiously represented chiefly in the form of well rounded vacuolar profiles These may occupy a considerable part of the cytoplasmic area In the myelo-

GRANULOCYTIC LEUKEMIA MICROGRAPHS

cyte stage it is somewhat reduced but still in the form of large round or oval profiles When the metamyelocyte stage has been reached there may be a few large profiles left but most have been reduced to the small circular or oval profiles characteristic of maturity, and their number is distinctly reduced

The number of mitochondria in developing granulocytes becomes gradually less from the promyelocyte stage to maturity However it is quite variable and constitutes only an incidental aid to judgment of the developmental status of the cell

In neutrophils the appearance of the specific granules changes with the stage of development When the myelocyte stage is reached with its full complement of granules, the granule size is noticeably larger than in mature cells In metamyelocytes many granules are small, although large ones may persist both here and in mature cells But in mature cells most of the granules are quite small

The development of eosinophilic and basophilic granules is vague be cause of the uncertain distinction between the two types Homogeneous granules the classic basophilic type are found predominantly in promyelocytes and early myelocytes Granules with internal ultrastruc ture, the eosinophilic type are found in myelocytes and metamyelo cytes It is therefore highly probable that whatever ultrastructure is acquired by the granules is developed in the transition from promyelo cytes to myelocytes There seems to be no significant change in the "eosinophilic granules as the metamyelocyte stage is reached How ever the basophilic bodies of eosinophils have not been observed at any stage earlier than metamyelocytes

Although developing cells of the erythrocytic series are occasionally seen in the granulocytic leukemias their characteristics are not described in detail here There is a short section on the erythrocytic series on pages 335 to 343

Studies of the electron morphology of thin sections of the cell types in granulocytic leukemia have been made by Bessis and Breton Gorius [2 3] Huth and Schneider [4] and Di Mayorca et al [5] A compre hensive review of the present status of this leukemia has been made by Bessis [1 chap X]

REFERENCES

1 Bessis M Cytology of the blood and blood forming organs Grune and Strat ton New York 1956
2 Bessis M and J Breton-Gorius Examen au microscope electronique des cellules des leucemies myeloides *Bull microscop appl* 5(1 2) 10–11 1955
3 Bessis M and J Breton Gorius Examen des cellules leucemiques au micro scope electronique par la methode des coupes *Presse med* 10 189–92 1955
4 Huth E and C Schneider Electron microscope appearance of normal leuco cytes and cells in acute leukosis in children *Ztschr Kind rh* 75 358–364 1954
5 Di Mayorca G G Lanzavecchia and A Le Coultre Studio sulla morfologia dei leucociti umane normale e leucemici col metodo della sezione sottili al microscopio elettronico *Rend Ist lombardo sci e lett* 90 559–572 1956

mia

GRANULOCYTIC LEUKEMIA MICROGRAPHS

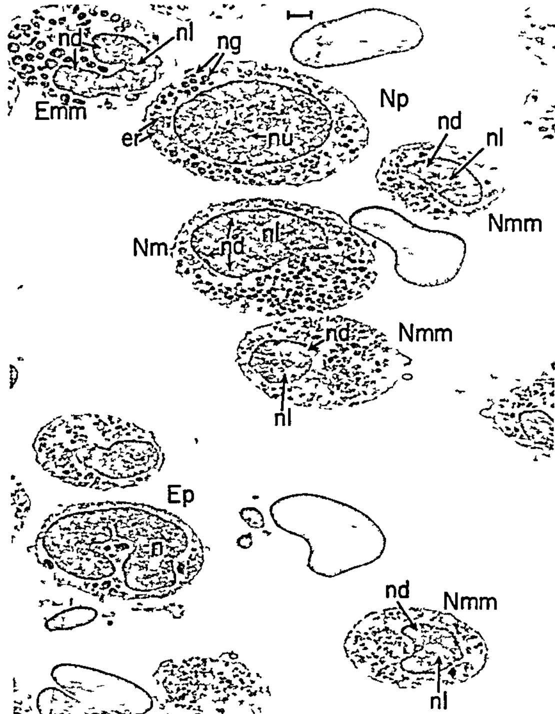
(Case 15 before treatment) 6800 X

Immature forms Cells of the neutrophilic series are labeled *N* and those of the eosinophilic series *E* with small letters indicating the probable stage of development *p*, promyelocyte *m* myelocyte and *mm* metamyelocyte

The cell at *Np* appears to be an early form and is probably a promyelocyte The granules (*ng*) are neutrophilic but few in number There are numerous profiles of endoplasmic reticulum (*er*) The nucleus is well rounded and there is a nucleolus (*nu*) The nucleoplasm is of fairly uniform density All these circumstances indicate an early stage of development Below this cell at *Nm*, is a cell of the neutrophilic series which is further advanced developmentally The granules are more numerous and there is less endoplasmic reticulum The nucleus is still fairly well rounded but has begun to show differentiation of nucleoplasm into dark (*nd*) and light (*nl*) areas This evidence indicates a neutrophilic myelocyte The other cells of the neutrophilic series labeled *Nmm* although not affording many clues have probably reached the metamyelocyte stage Supporting this interpretation is the apparently full complement of granules little endoplasmic reticulum and advanced development of nucleoplasm into light and dark areas in the pattern associated with mature cells (see pages 37 to 47)

Two cells of the eosinophilic series are present At *Emm* the granules are recognizable as being of the eosinophilic type because of their internal ultrastructure The full number seems to be present The nucleoplasm has differentiated into dark and light areas with characteristic patterning (pages 53 to 59) The nuclear shape suggests banding All these characteristics point to an eosinophilic metamyelocyte In the cell at *Ep the nucleoplasm (n) shows only the faintest indication of two* densities and the number of granules which possess internal ultrastructure is small Both these circumstances indicate an early stage The irregular contour of the nucleus is contradictory but nuclear shape in thin sections is a capricious criterion at best The evidence favors identification of this cell as an eosinophilic promyelocyte approaching the myelocyte stage

nia

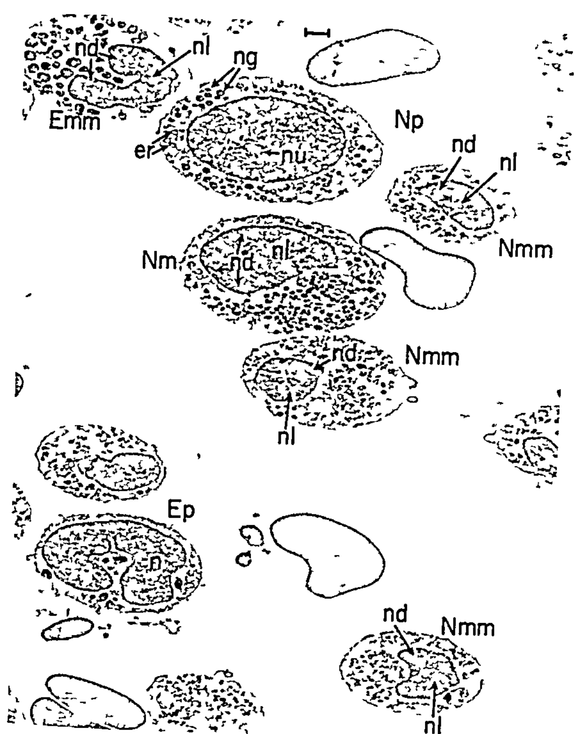

nd
nl
ng
Emm
Np
er
nu
nd
nl
Nmm
Nm
nd
nl
nd
Nmm
nl
Ep
n
nd
Nmm
nl

(Case 15 before treatment) 6800 X

Immature forms Cells of the neutrophilic series are labeled *N* and those of the eosinophilic series *E* with small letters indicating the probable stage of development *p* promyelocyte *m* myelocyte and *mm* metamyelocyte

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nd
nl
ng
Emm
er
nu
Np
nd
nl
Nmm
Nm
nd
nl
nd
Nmm
nl
Ep
n
nd
Nmm
nl



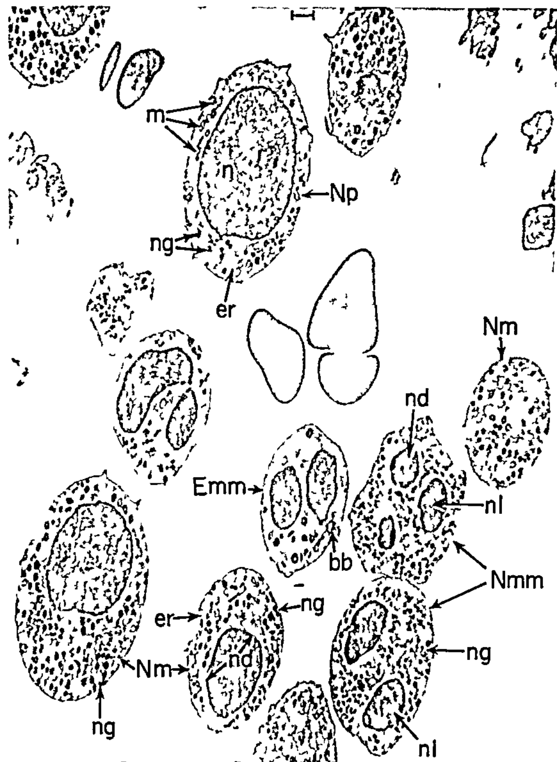
(Case 15 before treatment) 1100 X

Neutrophilic forms The neutrophilic promyelo cytes (*Np*) and the neutrophilic myelocyte (*Nm*) may be compared with the same developmental stages in the electron micrograph The banded neutrophil (*N*) is comparable to the cell in the lower right corner of the electron micrograph (See text at beginning of this section on termi nology) Myeloblasts (*MB*) are also present

(Case 15 before treatment) 5400 X

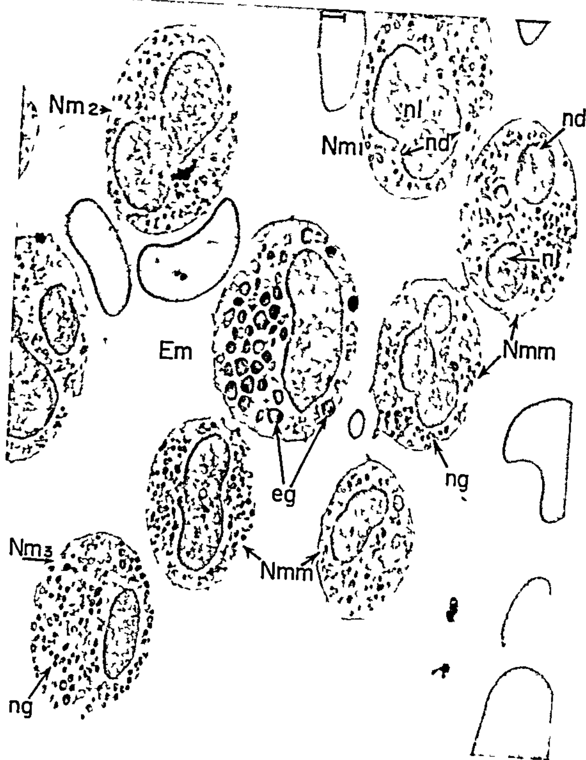
Neutrophilic forms The cell at *Np* is probably the most immature of the group as indicated by its undifferentiated nucleoplasm (*n*) numer ous large circular profiles of endoplasmic reticulum (*er*) sparse spe cific granules (*ng*) and fairly numerous mitochondria (*m*) This cell is a neutrophilic promyelocyte The next stage in line is represented by the two neutrophilic myelocytes (*Nm*) which have a greater number of large specific granules and fewer profiles of endoplasmic reticulum which are however large and round In the myelocyte at the right the nucleoplasm has undergone noticeable differentiation as indicated by the dark areas (*nd*) inside the nuclear membrane The two cells at *Nmm* have at least reached the metamyelocyte stage as evidenced by light (*nl*) and dark (*nd*) nucleoplasm in strong contrast numerous specific granules many of which are small and very little endoplasmic reticu lum The non nucleated portion of a cell at *Nm* is probably from a neutrophilic myelocyte for the reasons cited above The cell at *Emm* is probably a metamyelocyte of the eosinophilic series since a baso philic body (*bb*) is present

nia


m
n
Np
ng
er
Nm
nd
Emm
nl
bb
Nmm
er
ng
Nm
nd
ng
ng
nl

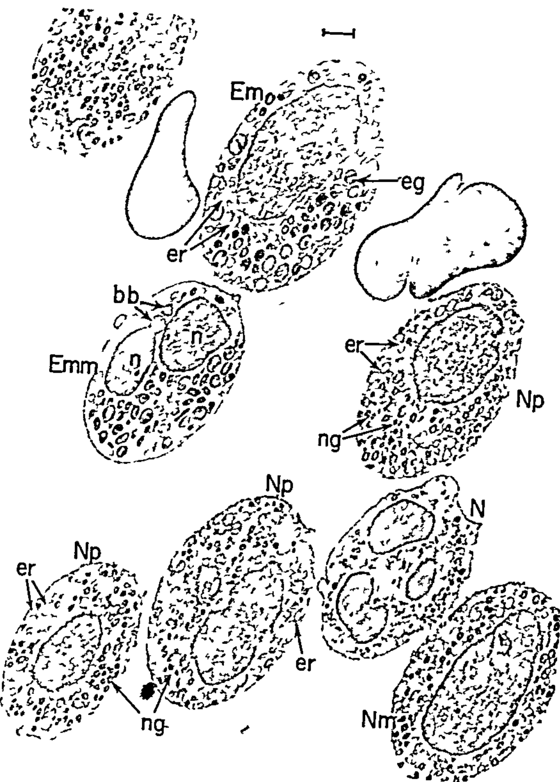
(Case 15 before treatment) 1100 X
Neutrophilic forms The neutrophilic promyelocytes (*Np*) and the neutrophilic myelocyte (*Nm*) may be compared with the same developmental stages in the electron micrograph The banded neutrophil (*N*) is comparable to the cell in the lower right corner of the electron micrograph (See text at beginning of this section on terminology) Myeloblasts (*MB*) are also present

(Case 15 before treatment) 5400 X
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Nm2
Nm1
nl
nd
nd
nl
Em
Nmm
eg
ng
Nm3
Nmm
ng

(Case 15 before treatment) 1100 X
Immature granulocytes The eosinophilic forms (*E*) may be compared with the eosinophilic mye locyte (*Em*) on the facing page The neutrophil (*N*) is slightly more advanced than the neutro philic metamyelocytes (*Nmm*) in the electron micrograph There are also a myeloblast (*MB*) and a neutrophilic promyelocyte (*Np*) present

ie 15 before treatment) 6500 X
ature granulocvtes The myelocyte stage in the neutrophilic group presented at *Nm*1 Nuclear differentiation into light (*nl*) and dark areas has begun but is not so pronounced as in the metamyelocytes *m*) The cells at *Nm*2 and *Nm*3 are apparently somewhat more nced than *Nm*1 but the nucleoplasm is still not so differentiated the metamyelocytes In general the neutrophilic granules (*ng*) arge in the myelocytes with a tendency for many but not all of to become small in the metamyelocytes and mature neutrophils cell at *Em* has probably reached the myelocyte stage There is in ion of developing ultrastructure within its granules (*eg*) of the type icteristic of the eosinophilic series


Em
eg
er
bb
n
n
Emm
er
Np
ng
Np
N
Np
er
er
ng
Nm

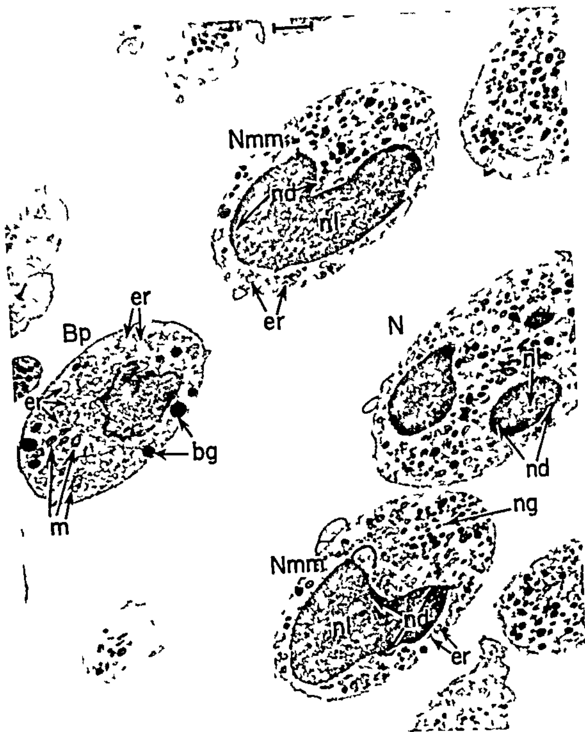
GENERAL FIELDS

(Case 15 before treatment) 8300 X

Various developmental stages of neutrophilic and eosinophilic series In the cell at *Em* the granules (*eg*) are eosinophilic and fairly numerous and there is abundant endoplasmic reticulum (*er*) The nucleus is well rounded and shows a definite tendency to two densities These features indicate an eosinophilic myelocyte The cell below (*Emm*) is somewhat more advanced as indicated by less endoplasmic reticulum and the two nuclear lobes (*n*) which are probably an expression of a banded nucleus passing twice through the plane of section Basophilic bodies (*bb*) which are present in this cell have not been observed at any stage earlier than metamyelocytes

Among the cells of the neutrophilic series those at *Np* are probably in the promyelocyte stage as indicated by copious endoplasmic reticulum (*er*) less than the full complement of specific granules (*ng*) and well rounded nuclei which have nucleoplasm of fairly even density The cell at *Nm* is further advanced and may be considered a neutrophilic myelocyte The cell at *N* is a mature neutrophil

nia


Nmm
nd
nl
er
N
nl
nd
Bp
er
er
bg
m
ng
Nmm
nl
nd
er

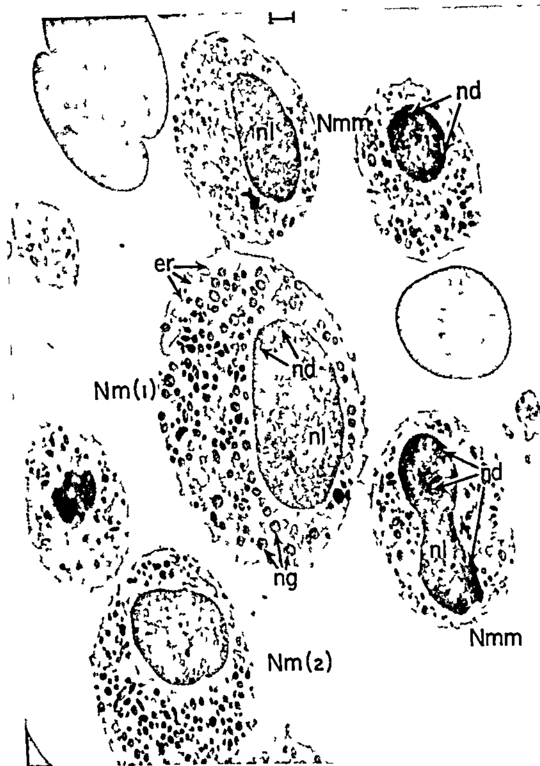
(Case 15 before treatment) 1100 X
Developing cells Two basophilic forms (*B*) are present but their exact stage of development is difficult to determine in light micrographs However the maturation of forms in electron micrographs can be more readily determined as on the facing page (*Bp*) The neutrophil (*N*) in the upper right corner may be compared with the neutrophil (*N*) in the electron micrograph

The myeloblast (MB), the neutrophilic pro myelocyte (*Np*), and the neutrophilic myelocyte (*Nm*) may be compared with the electron micrographs of these cell types presented on pages 149 to 157 221 223 251 to 255 and elsewhere

(Case 15 before treatment) 11 000 X
Developing cells There is one very early stage in the basophilic series (*Bp*) Several members of the neutrophilic series are further advanced The large basophilic granules (*bg*) are very sparse in the illustrated cell and mitochondria (*m*) are nearly as numerous Large profiles of endoplasmic reticulum (*er*) occupy the bulk of the cytoplasm All these characteristics point to a basophilic promyelocyte The only contradictory feature is the abnormally great density of the nucleoplasm which although not clearly showing differentiation into light and dark areas is much darker than usual for this stage of development

The members of the neutrophilic series are well advanced There are two typical metamyelocytes (*Nmm*) with numerous specific granules (*ng*) sparse endoplasmic reticulum (*er*) and well differentiated nucleoplasm clearly showing two densities light (*nl*) and dark (*nd*) The remaining neutrophilic form (*N*) has all the characteristics of a mature neutrophil (pages 37 to 47) Note that the two nucleoplasmic densities stand in somewhat higher contrast to each other than in the metamyelocytes

nia


nl
Nmm
nd
er
nd
Nm(1)
nl
nd
nl
ng
Nmm
Nm(2)

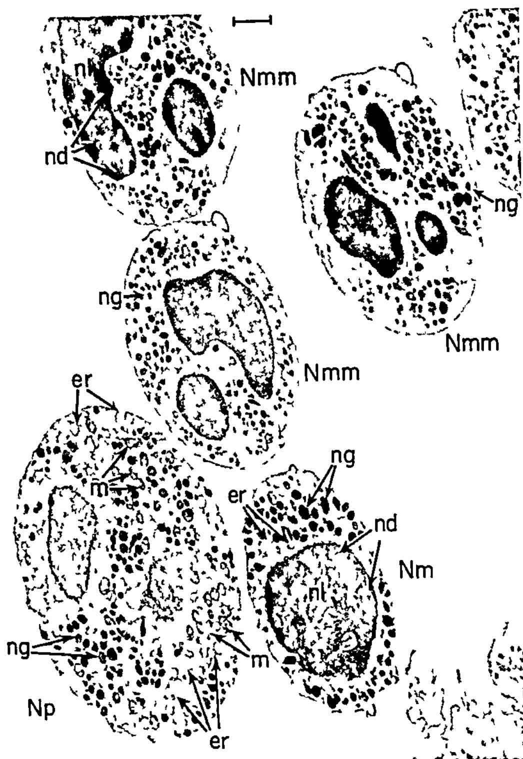
(Case 15 before treatment) 1100 X

Neutrophilic forms The two neutrophilic myelocytes (*Nm*) may be compared with the same cell type in the electron micrograph The neutrophil (*N*) with banded nucleus may be compared with banded neutrophilic forms (*Nmm*) in the electron micrograph Myeloblasts (*MB*) are present

(Case 15 before treatment) 6700 X

Neutrophilic forms The cell at *Nm*(1) is a typical neutrophilic myelocyte The specific granules (*ng*) are large and numerous and large profiles of endoplasmic reticulum (*er*) may be seen The nucleoplasm shows an early stage of differentiation into light (*nl*) and dark (*nd*) areas The cell at *Nm*(2) is further advanced as shown by smaller specific granules and more advanced nucleoplasmic differentiation The three cells labeled *Nmm* have reached the metamyelocyte stage with small specific granules and still higher contrast between the light and dark nucleoplasmic areas

mia

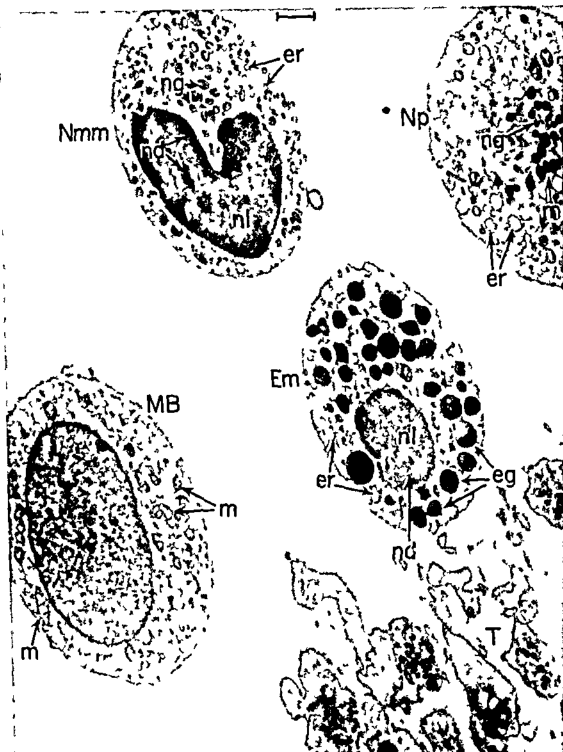

nl
nd
Nmm
ng
ng
Nmm
Nmm
er
m
ng
er
nd
Nm
nl
ng
m
Np
er

(Case 15 before treatment) 1100 X
Neutrophilic maturation The two neutrophilic promyelocytes (*Np*) the neutrophilic myelocyte (*Nm*) and the two neutrophilic metamyelocytes (*Nmm*) may be compared with the same forms in the electron micrograph on the facing page

(Case 15 before treatment) 10 000 X
Neutrophilic maturation The cell at *Np* shows well the characteristics of a neutrophilic promyelocyte The specific granules (*ng*) are large *but not numerous and there are abundant large profiles of endoplasmic* reticulum (*er*) Numerous mitochondria (*m*) are visible Although very little of the nucleus appears in this section the nucleoplasm is largely undifferentiated

The cell at *Nm* is a neutrophilic myelocyte The specific granules (*ng*) are large and numerous Light (*nl*) and dark (*nd*) areas can be distinguished *in the nucleoplasm At this stage large profiles of endo* plasmic reticulum are normally visible but there are not many in this section probably because of the small amount of cytoplasm present

The three cells labeled *Nmm* show the characteristics of neutrophilic metamyelocytes but there is nothing to distinguish them from mature neutrophils except their nuclear morphology which suggests a banded rather than a polymorphous structure Note that although the specific granules tend to be smaller there is considerable variation in size The two nuclear densities are well contrasted and their pattern is mature



er
ng
Nmm
nd
nl
Np
ng
m
er
Em
MB
nl
m
er
eg
nd
m
T

(Case 15 before treatment) 10 000 X

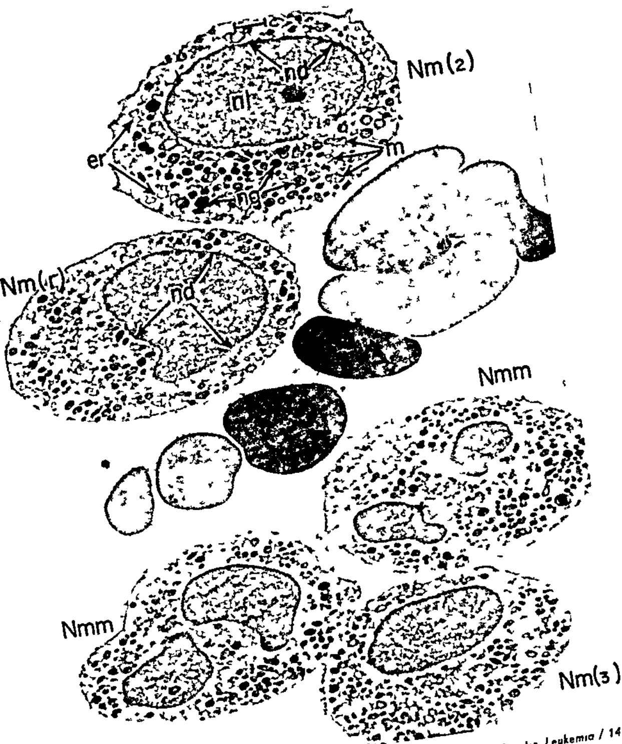
Diverse cell forms Distinctly different cell types are illustrated in this field At *Nmm* there is a neutrophilic metamyelocyte The mature number of granules (*ng*) are present and the endoplasmic reticulum (*er*) is present but reduced from earlier stages so that the profiles are small The nucleus is banded and its two densities (*nl nd*) are well developed

At *Np* there is a portion of a cell which appears to be a neutrophilic promyelocyte There are granules (*ng*) which appear to be neutrophilic but which are not all clearly distinguishable from nearby mitochondria (*m*) having unusually dense matrices The copious endoplasmic reticulum (*er*) with large profiles suggests an early form

At *Em* is an eosinophilic form which may be either a myelocyte or a metamyelocyte The granules (*eg*) are clearly eosinophilic and numerous The endoplasmic reticulum is more abundant than in mature forms *The nucleus shows both light and dark areas Its size however* suggests a single cut through a banded nucleus

The cell at *MB* is a myeloblast Numerous mitochondria (*m*) are present in a speckled cytoplasm The nucleus is of even contour and has nucleoplasm of even density This cell type is unusual in the granulocytic leukemias It is described in greater detail in the section on myeloblastic leukemia (pages 195 to 233)

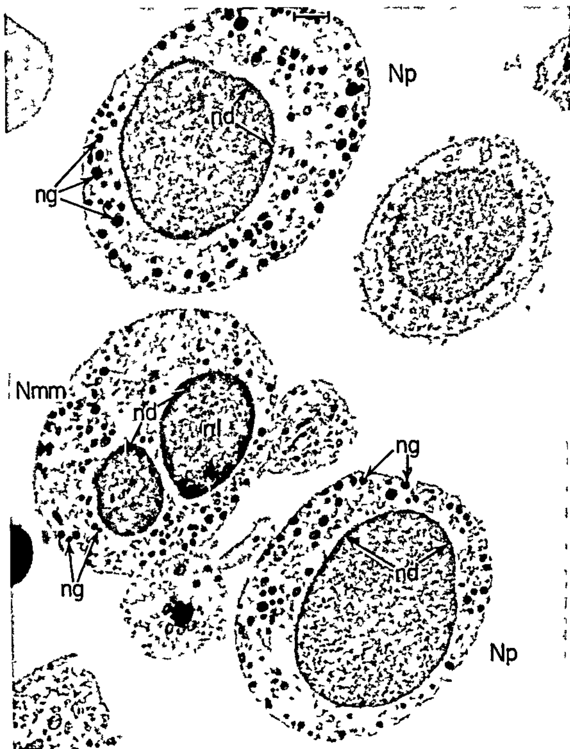
At *T* there is a typical group of blood platelets


Nm(2)
nd
nl
er
m
ng
Nm(1)
nd
Nmm
Nmm
Nm(3)

(Case 15 before treatment) 8700 X

Neutrophilic series The cells labeled *Nm* are not beyond the myelocyte stage The small number of large granules in *Nm*(1) suggests a very early stage but the differentiation of the nucleoplasm into dark areas around the nuclear membrane (*nd*) suggests that it is later than a pro myelocyte At *Nm*(2) all the characteristics suggest a myelocyte nu merous large granules (*ng*) and large profiles of endoplasmic reticulum (*er*) fairly numerous mitochondria (*m*) and nucleoplasm in established differentiation into light (*nl*) and dark (*nd*) areas The cell at *Nm*(3) presents much the same features but there are fewer mitochondria a not unusual circumstance which does not interfere with its identifica tion as a neutrophilic myelocyte The cells at *Nmm* are in the meta myelocyte stage as indicated by large numbers of neutrophilic granules many of which are small absence of large profiles of endoplasmic re ticulum and well established nucleoplasmic differentiation in nuclei of irregular contour

emia

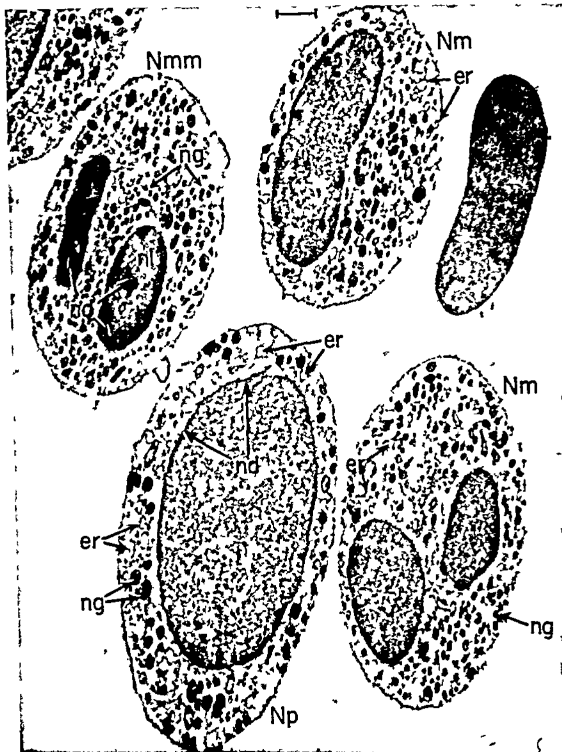

Np
nd
ng
Nmm
nd
nl
ng
ng
nd
Np

(*Case 18a before treatment*) 9800 X

Atypical neutrophilic forms The cells at *Np* are in the promyelocyte stage The specific granules (*ng*) are sparse and the nucleoplasm is largely of uniform density There are however thin bands of dark nucleoplasm (*nd*) at the nuclear membranes an indication that the myelocyte stage is being approached These cells are atypical in that profiles of endoplasmic reticulum large or small are nearly absent and there are no mitochondria Also the neutrophilic granules are rather small for this stage of development Nevertheless there is little doubt that they are neutrophilic promyelocytes

The cell at *Nmm* shows a nucleoplasmic differentiation into light and dark areas which is characteristic of a late (*meta* or mature) stage of development But the granules (*ng*) are remarkably sparse Because the differentiation of nucleoplasmic densities is the most reliable single criterion in determining the stages of development this cell is interpreted to be a neutrophilic metamyelocyte

emia


Nmm
Nm
er
ng
nl
nd
er
Nm
nd
er
er
ng
ng
Np

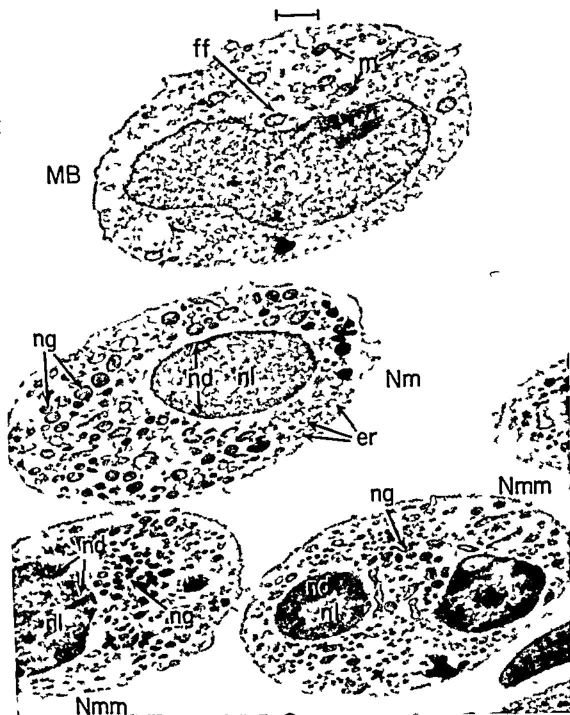
(Case 15 before treatment) 11 000 X

Progressive stages of differentiation of neutrophils The cell at *Np* is in an early stage The specific granules (*ng*) are large but not numerous Endoplasmic reticulum (*er*) is plentiful in large profiles The smooth contoured nucleus shows only the slightest indication of differentiation in the form of a thin dark band of nucleoplasm (*nd*) at the nuclear membrane All these features point to a neutrophilic promyelocyte

The two cells at *Nm* are further advanced and are in about the same stage The granules are smaller and the endoplasmic reticulum still has large profiles but fewer of them The nucleoplasm is clearly differentiated into light and dark areas but their contrast is not so heavy as in the metamyelocyte at upper left These cells are neutrophilic myelocytes

In the neutrophilic metamyelocyte (*Nmm*) the granules are numerous and although large ones are present they are in general small Large profiles of endoplasmic reticulum are scarce The nucleoplasm shows high contrast between its light and dark areas

emia



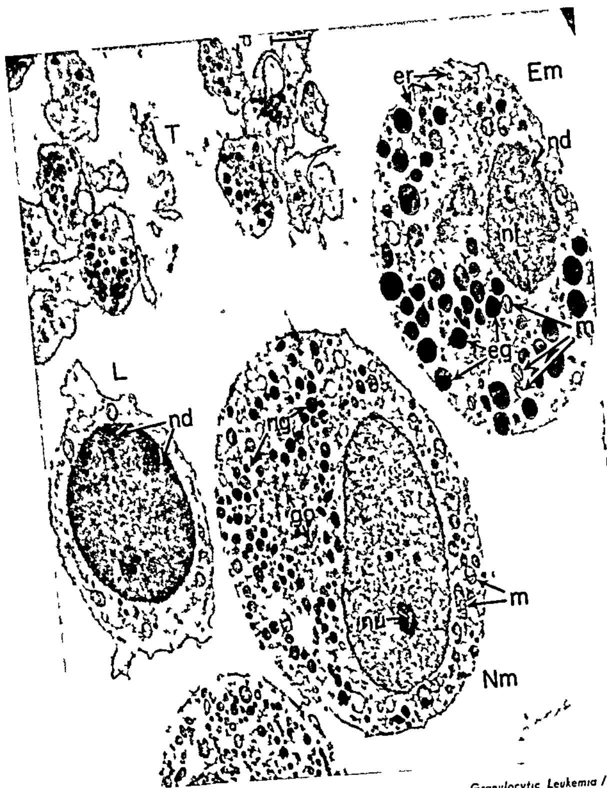
ff
m
MB
ng
nd
nl
Nm
er
Nmm
ng
nd
nl
ng
nd
nl
Nmm

(Case 15 before treatment) 12 000 X

Contrasting forms The cell at *MB* shows the distinguishing features of a monoblast Numerous small mitochondria (*m*) are distributed through out the characteristically speckled cytoplasm A fibrillar formation (*ff*) is visible The nucleus possesses a nucleolus and the nucleoplasm shows very little if any differentiation into the light and dark areas characteristic of the mature monocyte

At *Nm* the neutrophilic myelocyte is typical The granules (*ng*) are numerous and the endoplasmic reticulum (*er*) is plentiful in large pro files Differentiation of the nucleoplasm into light (*nl*) and dark (*nd*) areas is evident The two neutrophilic metamyelocytes (*Nmm*) have numerous but somewhat smaller specific granules (*ng*) and large pro files of endoplasmic reticulum are absent The nucleoplasm is well dif ferentiated into light and dark areas The cell at bottom right could be a mature neutrophil

mia



T
er
Em
nd
nl
m
eg
L
nd
ng
go
m
nu
Nm

(Case 15 before treatment) 10 000 X

Myelocytes and lymphocyte The cell labeled *Em* is an eosinophilic myelocyte The granules (*eg*) show typical internal ultrastructure and the endoplasmic reticulum (*er*) is abundant with profiles somewhat larger than those characteristic of mature cells Mitochondria (*m*) are present The nucleus shows moderate differentiation of its nucleoplasm into light (*nl*) and dense (*nd*) areas At *Nm* there is a typical neutrophilic myelocyte Numerous specific granules (*ng*) and large profiles of endoplasmic reticulum are present There are also mitochondria and a Golgi zone (*go*) The nucleus is still well rounded and possesses a nucleolus (*nu*) and differentiation of the nucleoplasm into light and dark areas has barely begun Although the nuclear morphology indicates a very early stage the number of neutrophilic granules shows that the cell is at least beyond the promyelocyte stage Their uniform large size indicates that this cell has not yet reached the metamyelocyte stage at which many of the neutrophilic granules become small

The cell at *L* possesses cytoplasm that is characteristic of a mature lymphocyte But its nuclear densities with the dark nucleoplasm (*nd*) very thick and chiefly near the nuclear membrane is not quite typical of the usual pattern (see pages 64 to 77)

A group of typical blood platelets is centered about *T* Note the close resemblance of their granules to the neutrophilic ones

emia

nd
er
nl
Nm
ng
er
N
er
er
eg
nl
E
nd
er
ng
bb
Nmm
nl
nd

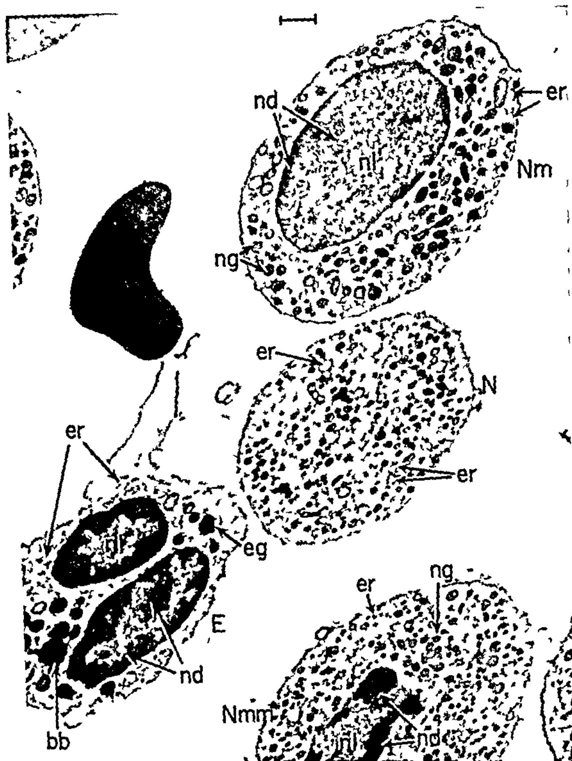
(Case 15 before treatment) 10 000 X

Late and mature forms The cell at *Nm* is a neutrophilic myelocyte The neutrophilic granules (*ng*) are characteristic and the endoplasmic reticulum (*er*) shows profiles that are in general somewhat larger than those in mature cells The nucleus is of smooth contour and differentiation of the nucleoplasm into light (*nl*) and dark (*nd*) areas is well established This cell may be compared with an earlier form on page 163 and with the later form (*Nmm*) in the same micrograph Identification of the neutrophilic metamyelocyte (*Nmm*) depends chiefly on the well advanced differentiation of the nucleoplasm into light and dark areas Also there is the full complement of neutrophilic granules many of which are small The endoplasmic reticulum where present is in the form of very minute profiles This cell could be a mature neutrophil

At *N* there is a cell of the neutrophilic series whose developmental status cannot be determined with certainty because the nucleus does not appear in the plane of section The full complement of neutrophilic granules many of them small indicates that it has reached the metamyelocyte stage but the large profiles of endoplasmic reticulum make it unlikely that maturity has been attained

The cell at *F* has the characteristics of a mature eosinophil There are eosinophilic granules (*eg*) and a basophilic body (*bb*) The endoplasmic reticulum appears as minute profiles The nuclear lobes show mature differentiation into light and dark nucleoplasm

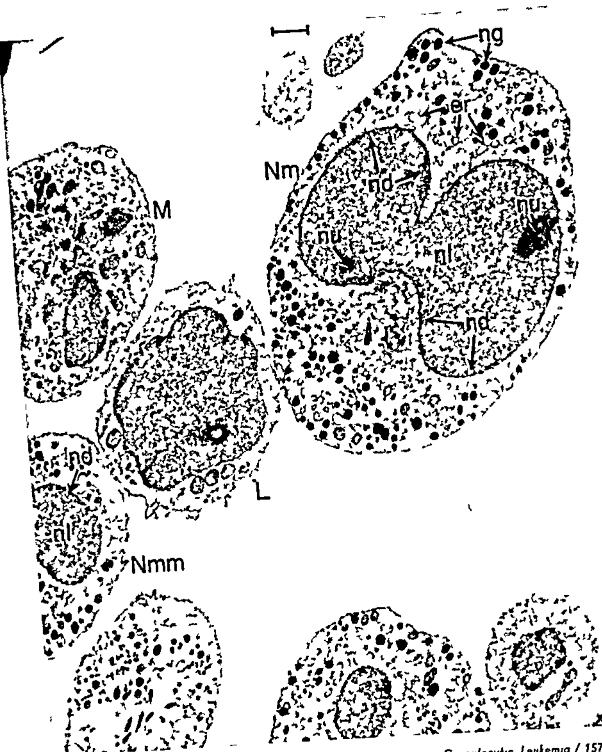
emia


nd
er
nl
Nm
ng
er
N
er
er
eg
nl
E
nd
ng
er
bb
Nmm
nl
nd

(Case 18a before treatment) 9800 X

Mixed cell group The large cell at *Nm* is a neutrophilic form which appears to be about midway in its progress from promyelocyte to myelocyte Its granules (*ng*) are clearly neutrophilic but they are not quite so numerous as might be expected if the full complement were present There are numerous profiles of endoplasmic reticulum (*er*) and these are large as is characteristic of immature forms Two nucleoli (*nu*) are present and the nucleoplasm shows the earliest recog nizable stage of differentiation into light (*nl*) and dark (*nd*) areas the latter collected under the nuclear membrane The constricted form of the nucleus is not typical of this stage of development but never theless is not unusual Nuclear shape is a useful criterion in judging statistically adequate numbers of cells but variations of the usual form in individual cells are common Unusual nuclear shape should not interfere with identification of the cell according to indications pre sented by other available criteria

Among the other cells that at *L* is a mature lymphocyte (see pages 12 and 64 to 77 for criteria) and that at *M* is a mature monocyte (see pages 12 and 78 to 85 for criteria) At *Nmm* a portion of a neutrophilic metamyelocyte is visible and is so identified chiefly because of the ad vanced differentiation of the nucleoplasm into light and dark areas

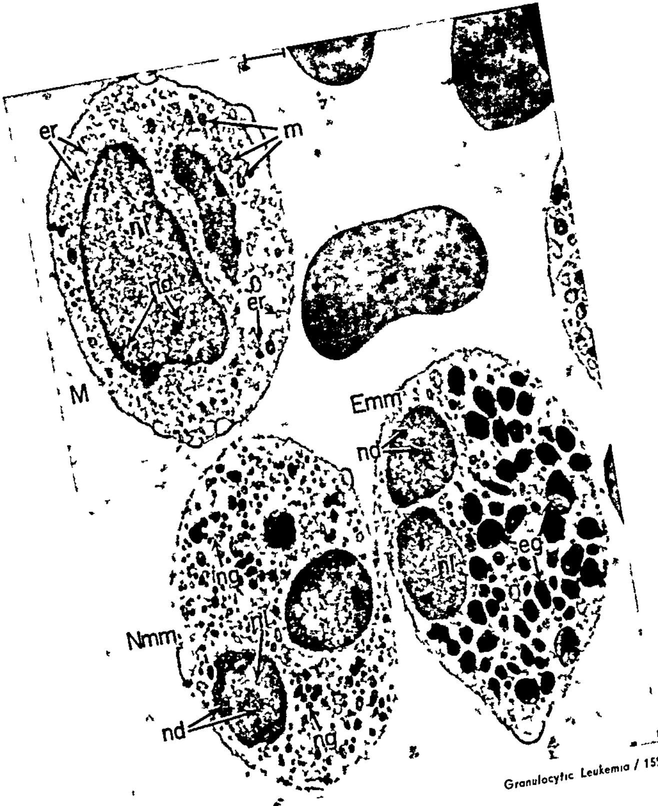

ng
er
Nm
nd
nu
nu
nl
nd
M
nd
nl
L
Nmm

(Case 15 before treatment) *11 000 X*

Maturing forms The cell at *M* is a mature monocyte It possesses small mitochondria (*m*) very small profiles of endoplasmic reticulum (*er*) generally speckled cytoplasm and two distinct densities in the nucleo plasm The darker nucleoplasm (*nd*) is against the nuclear membrane and to some extent deeper in the nucleus while the lighter portion (*nl*) occupies most of the nuclear body This cell may be compared with other mature forms illustrated on pages 78 to 85

The remaining two cells are metamyelocytes neutrophilic (*Nmm*) and eosinophilic (*Emm*) Both have the mature number of granules (*ng eg*) sparse endoplasmic reticulum and light and dark nucleoplasm *There is no certain way to distinguish these two cells from mature ones*

na

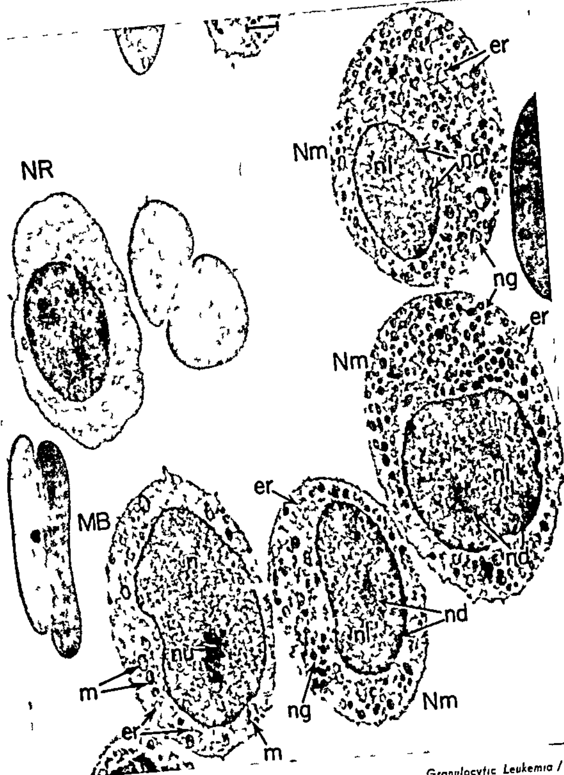

er
m
nl
ng
er
M
Emm
nd
seg
nl
ring
nl
Nmm
nd
ng

(Case 15 before treatment) 9000 X

Myeloid and erythroid forms This field contains three cells of the neutrophilic series which are in the myelocyte stage (*Nm*) In each there are numerous neutrophilic granules (*ng*) and some endoplasmic reticulum (*er*) The nuclei in all three have developed light (*nl*) and dark (*nd*) nucleoplasm and are still fairly well rounded On the basis of the development and pattern of nucleoplasmic densities which are the most reliable criteria for judging developmental progress in these preparations the uppermost cell is probably the youngest the middle one somewhat further along and the lowermost one the most mature (pages 125 and 126)

The cell labeled *MB* has an even nuclear density indicating an early stage and a nucleolus (*nu*) There are no specific granules but mitochondria (*m*) and endoplasmic reticulum (*er*) are present It is interpreted to be a myeloblast although cells of this very primitive stage are seldom seen in the granulocytic leukemias

A nucleated red blood cell (*NR*) is present Its appearance is typical of erythroblasts at about the middle stage of development This stage of erythrocytic development is more completely described on page 342



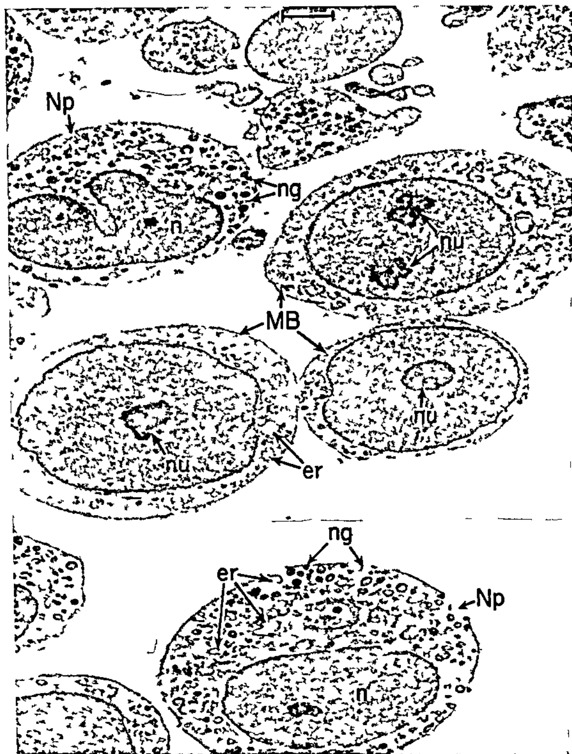
er
Nm
nl
nd
NR
ng
er
Nm
nl
nd
er
MB
nu
nd
nl
m
ng
Nm
er
m

(Case 15 before treatment) 9000 X

Myeloid and erythroid forms This field contains three cells of the neutrophilic series which are in the myelocyte stage (*Nm*) In each there are numerous neutrophilic granules (*ng*) and some endoplasmic reticulum (*er*) The nuclei in all three have developed light (*nl*) and dark (*nd*) nucleoplasm and are still fairly well rounded On the basis of the development and pattern of nucleoplasmic densities which are the most reliable criteria for judging developmental progress in these preparations the uppermost cell is probably the youngest the middle one somewhat further along and the lowermost one the most mature (pages 125 and 126)

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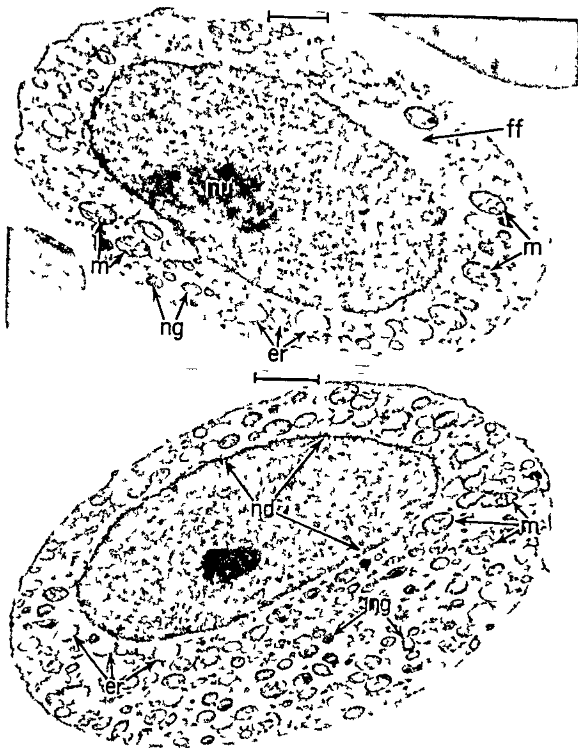

Np
ng
n
nu
MB
nu
nu
er
ng
er
Np
n

(Case 18b during treatment) 13 000 X

Unusually early cell types This field is a rather unusual one for a granulocytic leukemia since it shows cells in the very early stages of development These immature cells are more typical of myeloblastic leukemia

The three cells labeled *MB* are myeloblasts Nucleoli (*nu*) are prominent and the nucleoplasm is not clearly differentiated into light and dark areas The cytoplasm is free of specific granules and there is some endoplasmic reticulum (*er*) These cells may be compared with the myeloblasts described under myeloblastic leukemia (pages 198 to 211) The two members of the neutrophilic series present are both in early stages of differentiation At *Np* (below) the cell has few specific granules (*ng*) abundant endoplasmic reticulum (*er*) and largely un differentiated nucleoplasm (*n*) These characteristics all point to the promyelocyte stage At *Np* (above) the cell may be further advanced but neither the number of specific granules nor the slightly differentiated nucleoplasm indicate that it has passed the promyelocyte stage

nia


ff
nu
m
m
ng
er
nd
m
ng
er

NEUTROPHILIC FORMS

Upper (Case 15 before treatment) 16 000 X

Neutrophilic promyelocyte There is a small number of specific granules (*ng*) and large oval or circular profiles of endoplasmic reticulum (*er*) There are numerous mitochondria (*m*) and a nucleolus (*nu*) is present The nucleoplasm is not definitely differentiated Of particular interest is the fibrillar formation (*ff*) which although found in monoblasts in monoblastic leukemias has been observed in immature granulocytes in granulocytic leukemias

Lower (Case 15 before treatment) 18 000 X

Neutrophilic (pro)myelocyte This cell is a member of the neutrophilic series as shown by the specific granules (*ng*) They are large and fairly numerous but they do not seem to represent the full complement Endoplasmic reticulum (*er*) is represented by large oval or round profiles and there are many mitochondria (*m*) The nucleoplasm shows the beginning of differentiation in the form of a dark irregular band (*nd*) inside the nuclear membrane The characteristics of this cell indicate that it is midway between a promyelocyte and a myelocyte

mia

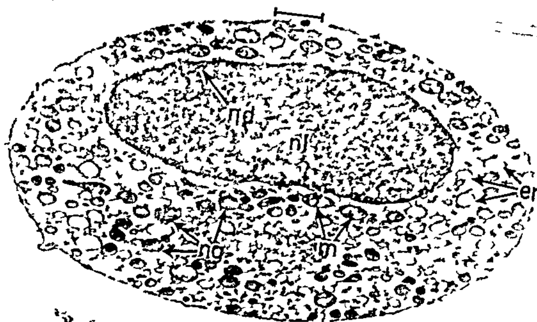
Neutrophilic myelocytes

Upper (Case 15 before treatment) 17 000 X

This member of the neutrophilic series has a population of specific granules (*ng*) that is at or near the full complement The definite but not strong differentiation of the nucleoplasm into light (*nl*) and dark (*nd*) areas indicates the myelocyte stage The large profiles of endo plasmic reticulum (*er*) fit this interpretation This cell is not far past the last phases of the promyelocyte stage

Lower (Case 15 before treatment) 13 000 X

This cell seems to be in about the same stage of development as the one above The specific granules (*ng*) are numerous and the nucleo plasm shows indication of differentiation into light (*nl*) and dark (*nd*) areas somewhat less marked than in the cell above The mitochondria (*m*) are more numerous as is usually the case in myelocytes and the large profiles of endoplasmic reticulum (*er*) are also more numerous

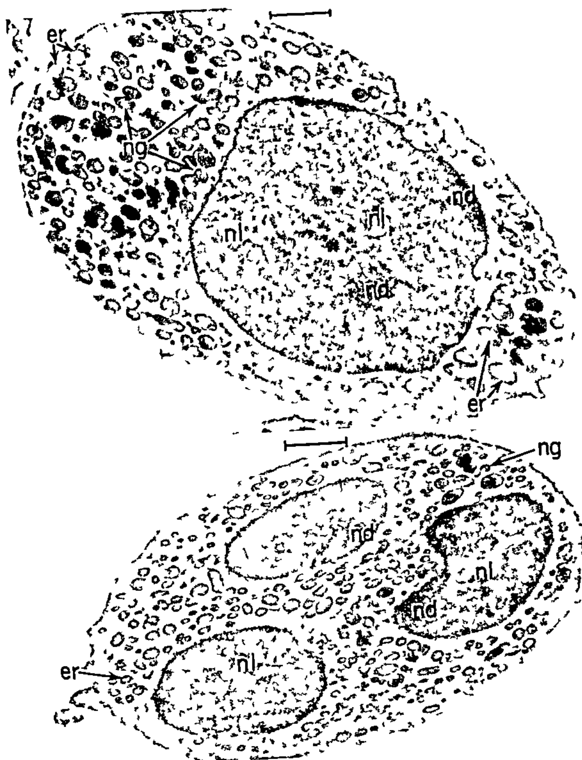

nd
nl
er
ng
m

Upper (Case 15 before treatment) **16 000 X**

Neutrophilic myelocyte The cytoplasmic characteristics are typical with a large number of neutrophilic granules (*ng*) and a moderate number of large profiles of endoplasmic reticulum (*er*) The nucleoplasm has differentiated into light (*nl*) and dark (*nd*) areas but its distribution in this cell is not entirely typical It may be compared to the usual pattern which is well illustrated on page 151

Lower (Case 15 before treatment) **17 000 X**

Mature neutrophil There is nothing in its make up to indicate that it is still in a developmental stage Neutrophilic granules (*ng*) are numerous and of varied size Endoplasmic reticulum (*er*) is sparse and is present mainly as very small profiles The nucleoplasm is well differentiated into light (*nl*) and dark (*nd*) areas in a mature pattern The three nuclear areas are about the size of the lobes of a polymorphous nucleus


er
ng
nl
nd
nl
nd
er
ng
nd
nl
nd
nl
er

Neutrophilic metamyelocytes

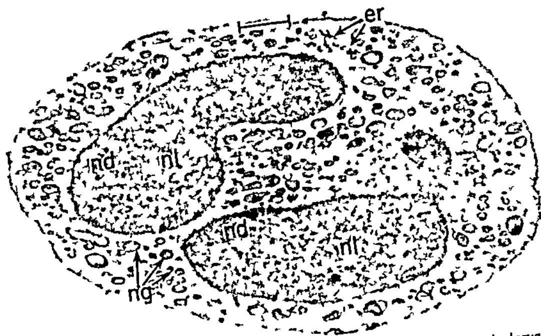
Upper (Case 15 before treatment) 18 000 X

The specific granules (*ng*) are small and numerous Large profiles of endoplasmic reticulum have mostly disappeared The nucleoplasm has differentiated strongly into light (*nl*) and dark (*nd*) areas with characteristic patterning (page 141) The rather harsh contrast between the two as compared to the softer tones of the micrograph below is a peculiarity of the preparation The nucleus is typically banded

Lower (Case 15 before treatment) 14 000 X

All the features are characteristic numerous neutrophilic granules (*ng*) moderate endoplasmic reticulum (*er*) mostly in small profiles differentiated nucleoplasm with characteristic pattern (*nl nd*) and two rather large nuclear areas suggesting cross sections of a banded nucleus bent back on itself

ukemia


er
nd
nl
nd
nl
ng

Upper (Case 15 before treatment) 15 000 X

Member of neutrophilic series well along in development The chief indication of this is the clear differentiation of the nucleoplasm into light (*nl*) and dark (*nd*) areas Although the nucleus appears to be lobulated the group of rather large profiles of endoplasmic reticulum (*er*) argues against its being a mature neutrophil Note the long (1 3 μ) mitochondrion (*m*) At *ng* there is a rod shaped neutrophilic granule about 0 8 μ long

Lower (Case 15 before treatment) 19 000 X

Neutrophilic metamyelocyte This cell of the neutrophilic series is well differentiated as indicated by the large number of granules and the contrast between light (*nl*) and dark (*nd*) nucleoplasm Note the compound vacuole (*cv*) in the cytoplasm These structures are described under Normal Blood on page 116

m
ng
nd
nl
er
ind
nl
ind
cv

Upper (Case 15 before treatment) 18 000 X

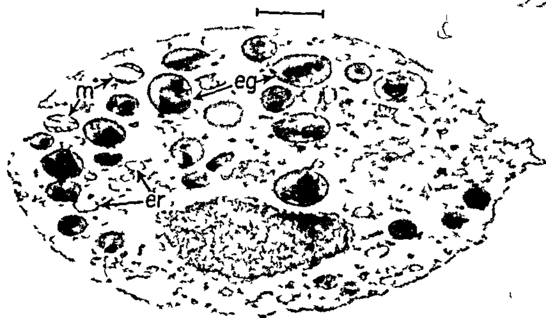
Cell entering myelocyte stage The eosinophilic granules (*eg*) are numer ous but may not quite represent the full complement Endoplasmic reticu lum (*er*) is abundant and the profiles vary considerably in size There are mitochondria (*m*) and a small portion of the Golgi zone (*go*) is visible The nucleoplasm is fairly well differentiated into light (*nl*) and dark (*nd*) areas

Lower (Case 15 before treatment) 19 000 X

Eosinophilic myelocyte Typical features are the full or nearly full number of specific granules (*eg*) the large profiles of endoplasmic reticulum (*er*) and the mitochondria (*m*) Because only a small portion of the nucleus is present it is not clear whether nucleoplasmic differentia tion is well established

emia

er nd er
eg
m nl
er go



EOSINOPHILIC FORMS

Upper (Case 15 before treatment) 18 000 X

Cell entering myelocyte stage The eosinophilic granules (*eg*) are numerous but may not quite represent the full complement Endoplasmic reticulum (*er*) is abundant and the profiles vary considerably in size There are mitochondria (*m*) and a small portion of the Golgi zone (*go*) is visible The nucleoplasm is fairly well differentiated into light (*nl*) and dark (*nd*) areas

Lower (Case 15 before treatment) 19 000 X

Eosinophilic myelocyte Typical features are the full or nearly full number of specific granules (*eg*) the large profiles of endoplasmic reticulum (*er*) and the mitochondria (*m*) Because only a small portion of the nucleus is present it is not clear whether nucleoplasmic differentiation is well established


er
m
er
nl
m
ind
eg
er
m
er
er
er
eg
ind
nl
er
m

Cells somewhat past middle stage of development in eosinophilic series

Upper (Case 15 before treatment) 15 000 X

The eosinophilic granules (*eg*) are typical There are no basophilic bodies Some mitochondria (*m*) are present Endoplasmic reticulum (*er*) is abundant some of it in small profiles but much of it large The nucleoplasm is well differentiated into light (*nl*) and dark (*nd*) areas This cell is very close to the metamyelocyte stage and may even have reached it Signs of immaturity are the large number of mitochondria numerous large profiles of endoplasmic reticulum and absence of baso philic bodies

Lower (Case 15 before treatment) 13 000 X

This cell has reached the myelocyte stage as indicated by the abundance of typical eosinophilic granules (*eg*) and the recognizable although not advanced differentiation of the nucleoplasm into light (*nl*) and dark (*nd*) areas However the fairly numerous mitochondria (*m*) and the abundant large profiles of endoplasmic reticulum (*er*) both indicate an immature stage Differentiation in this cell has not progressed so far as in the cell above

Late stages in eosinophilic development

Upper (Case 15 before treatment) 18 000 X

This cell shows practically all the features of maturity The eosinophilic granules (*eg*) are numerous and show the typical inclusions somewhat denser than the remainder of the granule Basophilic bodies (*bb*) which have not been observed in any developmental stage earlier than meta myelocytes are present The endoplasmic reticulum (*er*) is abundant chiefly in the form of the small profiles which characterize the mature eosinophil but there are one or two profiles of larger size which indicate immaturity The Golgi zone (*go*) is present The nucleoplasm shows ad vanced differentiation into light (*nl*) and dark (*nd*) areas and cannot be distinguished from the mature nucleus In fact the only indications of immaturity in this cell are the large profiles of endoplasmic reticulum which can hardly be regarded as decisive evidence for incomplete de velopment

Lower (Case 15 before treatment) 18 000 X

This cell shows signs of being less advanced than the one above although it has definitely reached the metamyelocyte stage The eosinophilic gran ules (*eg*) and basophilic bodies (*bb*) are characteristic There are many small profiles of endoplasmic reticulum (*er*) but also a number of large ones Mitochondria (*m*) are recognizable The two characteristic nuclear densities (*nl nd*) are conspicuous


er
eg
nd
go
er
er
nl
eg
ng

EOSINOPHILIC FORMS

(Case 15 before treatment) 22 000 X

Eosinophilic metamyelocyte This cell of the eosinophilic series has the full complement of specific granules (*eg*) and the endoplasmic reticulum (*er*) is reduced and chiefly present in the form of small profiles A Golgi zone (*go*) is present The nucleoplasm has both light (*nl*) and dark (*nd*) areas which although not in high contrast have a typical pattern (pages 53 to 59) They may be compared to the nucleoplasm of the neutrophilic nucleus at bottom right

kemia


er
er
eg
nd
go
eg
er
nl
ng

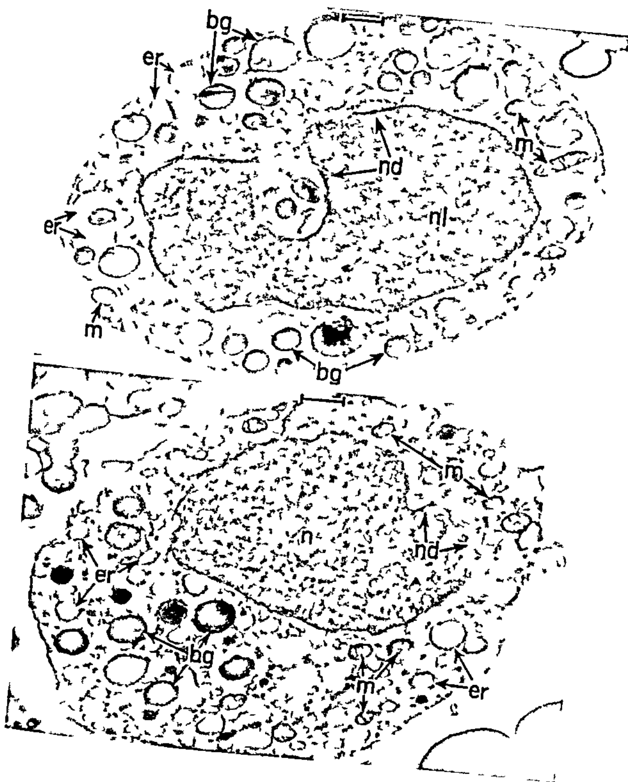
Early stages Despite certain ambiguities noted below both are interpreted to be members of the basophilic series

Upper (Case 15 before treatment) 12 000 X

The numerous large profiles of endoplasmic reticulum (*er*) indicate an early form but the specific granules (*bg*) are fairly abundant denoting some progress beyond the promyelocyte stage This is supported by the fact that the nucleoplasm has at least begun its differentiation into light (*nl*) and dark (*nd*) areas Mitochondria (*m*) are fairly numerous The evidence indicates a stage of maturation leaving the promyelocyte stage and entering the myelocyte stage There are irregularities of density in the specific granules However these do not conform to the classic pattern of the ultrastructure in eosinophilic granules The cell is interpreted to be a member of the basophilic series in the earliest myelocyte stage

Lower (Case 15 before treatment) 12 000 X

This basophilic form is in a somewhat earlier stage of development than the cell above The specific granules (*bg*) are basophilic without definite ultrastructure and are not numerous A definite membrane seems to surround some of them Both mitochondria (*m*) and large circular and oval profiles of endoplasmic reticulum (*er*) are abundant The nucleoplasm (*n*) is largely undifferentiated of even density with only a suggestion of darker nucleoplasm (*nd*) inside the nuclear membrane All these characteristics indicate that this cell is a basophilic promyelocyte

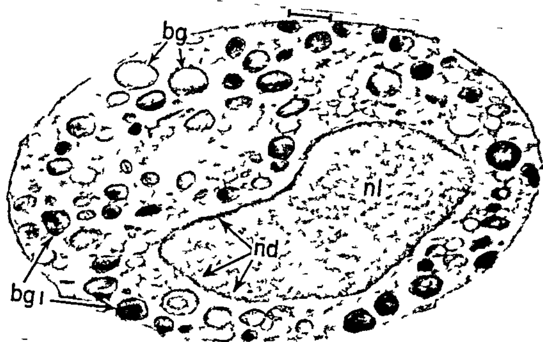

bg
bg
er
nd
m
m
nl
er
er
m
bg
m
n
nd
er
bg
m
er

BASOPHILIC FORMS

Basophilic promyelocytes Both these cells are in the early stages of de velopment and possess granules that are largely homogeneous They are interpreted to be basophilic promyelocytes although certain of their characteristics described below cast some doubt on this identification

Upper (Case 15 before treatment) **12 000 X**
The nucleus of this cell with only slight differentiation into light (*nl*) and dark (*nd*) nucleoplasm indicates an early stage The specific gran ules (*bg*) although numerous are homogeneous and therefore of the basophilic type But there is faint indication of developing ultrastruc ture within certain of them (*bg1*) The possible significance of this is discussed with the description of the cell below

Lower (Case 15 before treatment) **12 000 X**
This cell is also an early one as indicated by the slight nucleoplasmic differentiation into light (*nl*) and dark (*nd*) areas and also by the rather small number of specific granules (*bg*) Certain of the granules show faint developing internal ultrastructure (*bg1*) This may be the beginning of the development of the characteristic ultrastructure of eosinophilic granules No adequate criteria are presently available which would per mit a positive identification



bg1
bg1
bg
nd
nl

BASOPHILIC FORMS

Basophilic promyelocytes

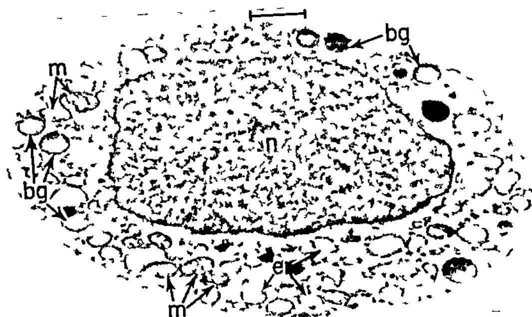
Upper (Case 15 before treatment) 14 000 X

This cell is a member of the basophilic series early in its developmental cycle The essentially homogeneous basophilic granules (*bg*) are conspicuous but not yet very numerous Note that the density of individual granules varies considerably Profiles of endoplasmic reticulum (*er*) are both large and numerous They tend to be a more conspicuous feature early in differentiation in the basophilic series than in comparable stages of the neutrophilic series Mitochondria (*m*) are present The nucleoplasm is largely undifferentiated with only a suggestion of a dark area (*nd*) inside a portion of the nuclear membrane

Lower (Case 15 before treatment) 16 000 X

This cell is in the same developmental stage as the one above Diagnostic features are a small number of largely homogeneous basophilic granules (*bg*) of varying density numerous large profiles of endoplasmic reticulum (*er*) mitochondria (*m*) and a nucleoplasm (*n*) that is largely undifferentiated

bg
er
m
nd
m
er


bg
m
bg
n
bg
er
m

(Case 15 before treatment) 24 000 X

Basophilic myelocyte The specific granules (*bg*) are typical and numer ous There is a membrane around the granules and some suggestion of an irregular pattern of ultrastructure within them Above and to the left in many of the granules there is a dense line which is a cutting artifact The direction of cut was down and to the right so that the dark portion of the granule was the first to come in contact with the knife This effect occurs whenever the fixed structures are unusually hard Endoplasmic reticulum (*er*) is abundant and the profiles are large in many cases Mitochondria (*m*) are present The nucleus shows well differentiated nucleoplasm with the light (*nl*) and dark (*nd*) areas in a typical pat tern The area in the center of the cytoplasm not occupied by basophilic granules may be due to a tangential cut through the Golgi zone but can not be positively identified

mia


m
bg
er
nd
nl
nd
nd
er

Upper (Case 15 before treatment) **16 000 X**

Basophilic promyelocyte This cell represents a fairly early developmental stage in the basophilic series The specific granules (*bg*) are characteristic but not yet numerous The endoplasmic reticulum (*er*) is abundant and the profiles are fairly large There are numerous mitochondria (*m*) The nucleoplasm is fairly even in its over all density but there is definite indication of a dark band (*nd*) under the nuclear membrane This cell is still in the promyelocyte stage but is close to a myelocyte

Lower (Case 15 before treatment) **22 000 X**

Basophilic myelocyte This member of the basophilic series is more advanced than the one above The basophilic granules (*bg*) are more numerous and are characteristic The profiles of endoplasmic reticulum (*er*) are very large and occupy a considerable portion of the cytoplasm Mitochondria (*m*) are present The nucleoplasm is clearly differentiated into light (*nl*) and dark (*nd*) areas


m
m
m
nd
er
bg
er
bg
er
m
nl
nd
er
bg

Basophilic myelocytes

Upper (Case 18b during treatment) 12 000 X

The nucleoplasm of this cell although not advanced in its differentiation definitely shows indication of light (*nl*) and dark (*nd*) areas Endoplasmic reticulum (*er*) is present in large profiles but is not abundant The basophilic granules (*bg*) are numerous and approach the full complement This cell is at least approaching the myelocyte stage

Lower (Case 18b during treatment) 16 000 X

The nucleoplasm of this cell shows definite signs of differentiating into light (*nl*) and dark (*nd*) areas Endoplasmic reticulum (*er*) is present in large profiles The basophilic granules (*bg*) are evident the large ones measuring about 1 μ in diameter Despite the rather small number of specific granules in this particular section the cell has probably entered the myelocyte stage

MYELOBLASTIC LEUKEMIA

In myeloblastic (acute myelogenous acute granulocytic) leukemia very immature cell types predominate Myeloblasts the most immature cells of the granulocytic and erythrocytic series are very common Promyelocytes myelocytes and early members of the erythrocytic series are also plentiful

The myeloblasts have the superficial appearance of agranulocytes but their immaturity is indicated by the undifferentiated character of their nucleoplasm In the true myeloblast the nucleus is large and the nucleoplasm very light and of even density throughout Endoplasmic reticulum is frequently seen in the form of flattened sacs (cisternae) As these cells *mature the nucleoplasm becomes darker and may show faint indication* of differentiation into light and dark areas although its full differentiation is not reached until late in the granulocytic or erythroblastic series Mitochondria become very numerous as the cytoplasmic volume increases but they decrease in number as maturation progresses through the specific series

The first recognizable signs of the transformation of myeloblasts into members of the granulocytic series is of course the appearance of specific granules Among neutrophilic forms the early granules are often quite difficult to distinguish from mitochondria because some *mitochon*dria seem to become very small with dense matrices at this stage The

transition from myeloblast into the earliest recognizable stage of the erythrocytic series is indicated by an increased density of both the undifferentiated cytoplasmic matrix and the nucleoplasm without any other visible change in the cell

At the promyelocyte stage the profiles of endoplasmic reticulum while large and copious have assumed round or oval shape in contrast to the cisternal form of the myeloblasts In the early stages of the erythrocytic series the cells have retained the cisternal form of the endoplasmic reticulum

Among the members of the granulocytic series neutrophilic forms prevail promyelocytes being the most common and metamyelocytes the least frequent Granules of the basophilic type (homogeneous) are seen in some promyelocytes which have been identified as basophilic although it is realized that they may be eosinophilic forms whose granules have not yet developed their ultrastructure

It is interesting to note that there is a close resemblance between the myeloblasts identified in the following pages and the monoblasts (pages 284 to 303) and lymphoblasts (pages 264 to 279) described in more detail in other sections It appears that the distinction between myeloblasts and the early blastic forms of agranulocytes is no easier in electron microscopy than in light microscopy

Electron micrographs of myeloblasts have been published by Bessis [1 chap X]

REFERENCE

1 Bessis M Cytology of the blood and blood forming organs Grune and Stratton New York 1956

MYELOBLASTIC LEUKEMIA MICROGRAPHS

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er
er
nu
m
m
er
nu
nu
m
m

(Case 20 before treatment) 9400 X

Myeloblasts This field illustrates four more or less typical cells of the chief type characteristic of myeloblastic leukemia They represent the cell preceding the specific cells of the granulocytic and erythroblastic series the myeloblast of the histologists Superficially they resemble agranulocytes but certain features notably the nuclear density mark them as immature cells and serve as a means of distinguishing them from mature lymphocytes and monocytes

The cytoplasm contains a large number of mitochondria (*m*) which are usually small both circular and rod shaped and more numerous than in lymphocytes or monocytes Those in the cell at upper right are larger than usual Endoplasmic reticulum (*er*) is present Many of the profiles are fairly large and many of them appear as elongate sacs a form which is not found in mature lymphocytes and monocytes The cytoplasmic matrix has a generally speckled appearance

The nuclei possess conspicuous nucleoli (*nu*) The nucleoplasm has an even over all density with little or no indication of light and dark areas This feature is the most reliable index of immaturity in these cells and contrasts markedly with the two densities characteristic of the nuclei of mature blood leukocytes including lymphocytes and monocytes Among the four cells here illustrated only the one at lower le t shows any indication of two densities in the nucleoplasm

:mia


er
er
er
ng
N
er
er
m
m
m

(Case 20 before treatment) **7200 X**

Myeloblasts The numerous mitochondria (*m*) are mostly small and circular The endoplasmic reticulum (*er*) is represented both as profiles of sacs and in circular form Nucleoli (*nu*) are conspicuous and the nucleoplasm is of even density indicating an undifferentiated form The cytoplasm at *N* has neutrophilic granules (*ng*) in it The large circular profiles of endoplasmic reticulum indicate that this cell is still immature

emia


MB
m
ng
MB
er
m
m
NR
g
MB

(Case 20 before treatment) 12 000 X

Myeloblasts (MB) and erythroblast (NR) The nuclei of the myeloblasts are largely undifferentiated although there is slight indication of a darker nucleoplasm (*nd*) inside the nuclear membrane The endoplasmic reticulum (*er*) in saclike formations is frequently seen in these cells Mitochondria (*m*) are quite numerous and there are no specific granules The cell at *NR* is an erythroblast Cells in the erythrocytic series are readily recognizable because both cytoplasm and nucleus are perceptibly darker than these structures in other cells Mitochondria are present and the cytoplasm contains a large number of granules (*g*) which have a matrix as dark as that of the undifferentiated cytoplasm The large number of cytoplasmic inclusions in this cell indicates that its differentiation has not progressed very far since these inclusions disappear as maturity is reached

mia


nd
nl
n
er
MB
er
er
er
n
nu
m

(Case 20 before treatment) 1100 X

Myeloblasts and neutrophilic promyelocyte (Np)
Note the large nuclei and scanty cytoplasm in the myeloblasts They may be compared with the cells in the electron micrograph on the facing page

(Case 20 before treatment) 11 000 X

Typical myeloblasts (MB) The nucleoplasm (*n*) in the cells at upper right and lower left is fairly even but in the cell at upper left there are distinct light (*nl*) and dark (*nd*) areas Certain structures in this cell could be either neutrophilic granules or mitochondria and it is therefore possible that it may have entered the neutrophilic promyelocyte stage The endoplasmic reticulum (*er*) of all three cells is characteristic The mitochondria (*m*) and saclike endoplasmic reticulum (*er*) of the mass of cytoplasm at lower right suggest a myeloblast Note the large complex nucleolus (*nu*) in the cell at lower left

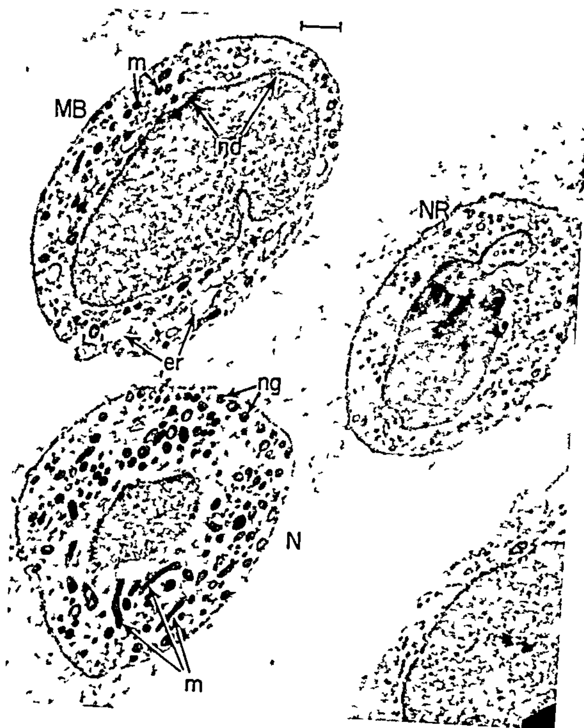

nd
nl
n
er
MB
er
er
er
n
nu
m

(Case 20 before treatment) 1100 X

Myeloblasts and neutrophilic promyelocyte (Np) Note the large nuclei and scanty cytoplasm in the myeloblasts They may be compared with the cells in the electron micrograph on the facing page

(Case 20 before treatment) 11 000 X

Typical myeloblasts (MB) The nucleoplasm (*n*) in the cells at upper right and lower left is fairly even but in the cell at upper left there are
: (*nd*) areas Certain structures in this cell
: granules or mitrochondria and it is there
iave entered the neutrophilic promyelocyte
:ulum (*er*) of all three cells is characteristic
saclike endoplasmic reticulum (*er*) of the
right suggest a myeloblast Note the large
he cell at lower left


m
MB
nd
er
ng
N
m
NR

(Case 20 before treatment) 11 000 X

Mixed cell group The myeloblast at *MB* has typical features Mito chondria (*m*) are numerous and the profiles of endoplasmic reticulum (*er*) are large The nucleoplasm is largely undifferentiated but there is suggestion of a dark area (*nd*) inside the nuclear membrane The large number of neutrophilic granules (*ng*) in the cell at *N* identifies its series but the remaining features are not sufficient to determine its stage How ever the large number of mitochondria suggest that it is not mature The cell at *NR* is a nucleated red blood cell Note the high density of both nucleus and cytoplasm as compared to the other cells The large number of cytoplasmic inclusions also suggests a fairly early stage

emia

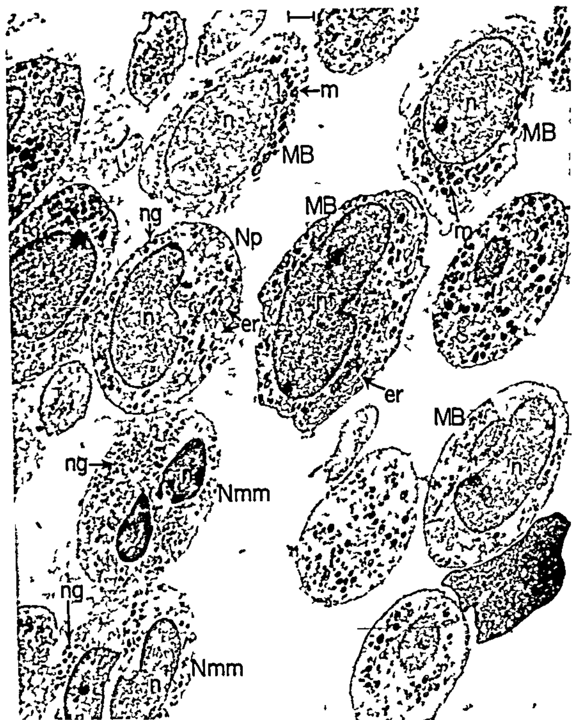


m
MB
nd
NR
er
ng
N
m

(Case 20 before treatment) **7300 X**

Myeloblasts (MB) and members of neutrophilic series Compare the nuclear and cytoplasmic densities in the four myeloblasts (*MB*) which in spite of the wide range of densities present all possess the characteristics of their cell type The nucleoplasm (*n*) in all of them is essentially undifferentiated Numerous small mitochondria (*m*) are characteristic The endoplasmic reticulum (*er*) varies considerably but its presence in the form of sacs is common The cell at *Np* is probably a neutrophilic promyelocyte but the specific granules (*ng*) are very sparse and difficult to identify with certainty Note the large oval profiles of endoplasmic reticulum (*er*) The nucleoplasm is undifferentiated There are also two neutrophilic metamyelocytes (*Nmm*) present with characteristically numerous small specific granules (*ng*) and well differentiated nucleoplasm (*n*) The nuclear shape suggests banding

nia


m
MB
n
MB
MB
ng
Np
m
n
n
er
er
MB
n
ng
Nmm
ng
Nmm
n

(Case 20 before treatment) 7300 X

Myeloblasts (MB) and members of neutrophilic series Compare the nuclear and cytoplasmic densities in the four myeloblasts (*MB*) which in spite of the wide range of densities present all possess the characteristics of their cell type The nucleoplasm (*n*) in all of them is essentially *undifferentiated Numerous small mitochondria (m) are characteristic* The endoplasmic reticulum (*er*) varies considerably but its presence in the form of sacs is common The cell at *Np* is probably a neutrophilic promyelocyte but the specific granules (*ng*) are very sparse and difficult to identify with certainty Note the large oval profiles of endoplasmic reticulum (*er*) The nucleoplasm is undifferentiated There are also two neutrophilic metamyelocytes (*Nmm*) present with characteristically numerous small specific granules (*ng*) and well differentiated nucleoplasm (*n*) The nuclear shape suggests banding

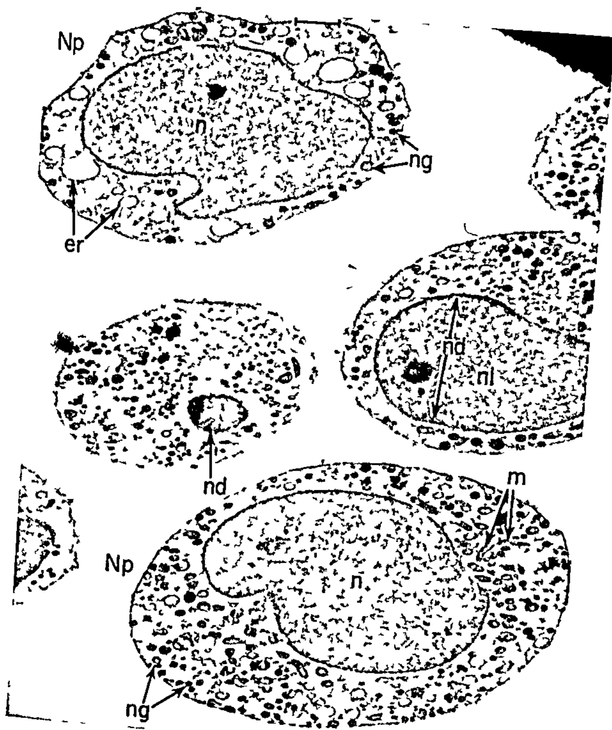
mia


er
n
ng
ng
Np
er
ng
MB
n
Np
ng
er
n
ff

(Case 20 before treatment) 11 000 X

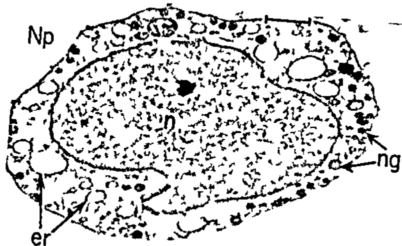
Early stages of differentiation These cells possess numerous mitochondria abundant endoplasmic reticulum (*er*) and undifferentiated nucleoplasm (*n*) of even density Three of them (*Np*) contain a small number of what appear to be neutrophilic granules (*ns*) and therefore are interpreted to be neutrophilic promyelocytes At *ff* there is what appears to be a fibrillar formation (pages 309 and 311) At *MB* the cell partially visible is a myeloblast

nia

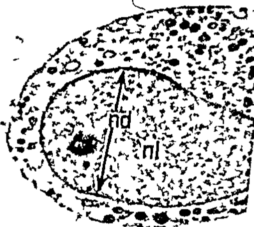

Np
n
ng
ng
er
nd
nl
nd
m
Np
n
ng

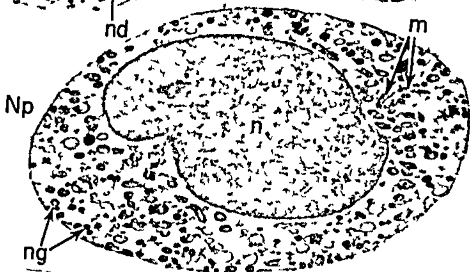
(Case 20 before treatment) 13 000 X

Neutrophilic maturation At *Np* (above) is a neutrophilic promyelocyte The specific granules (*ng*) are not yet numerous and the endoplasmic reticulum (*er*) is conspicuously large mostly in oval profiles The cell at *Np* (below) is also a neutrophilic promyelocyte but is further along in its development The specific granules are more numerous and mito chondria (*m*) are still plentiful Note the essentially undifferentiated nucleoplasm (*n*) in both these cells It is fairly even except for greater density near the nuclear membrane The nucleus of the neutrophilic cell at middle right is fairly clearly differentiated into light (*nl*) and dark (*nd*) areas This cell has developed to the point where it may be con sidered a myelocyte The cell at middle left is also neutrophilic and is even further along in its development as indicated by the very dense nucleoplasm in the small portion of the nucleus present


Np
n
ng
er


nd

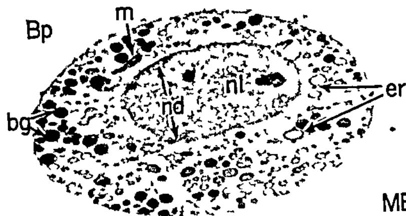

nd
nl

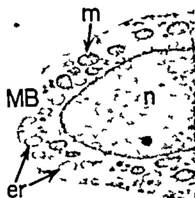

m
Np
n
ng

(Case 20 before treatment) **8800 X**

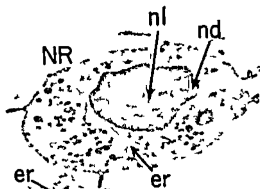
Diverse cell types At *Bp* there is a basophilic promyelocyte The granules (*bg*) are large and homogeneous but not numerous The endoplasmic reticulum (*er*) is chiefly found in the form of large round or oval profiles and mitochondria (*m*) are recognizable The nucleoplasm is fairly well differentiated into light (*nl*) and dark (*nd*) areas The cells at *MB* have the characteristics of myeloblasts Note particularly the undifferentiated nucleoplasm (*n*) The endoplasmic reticulum and mitochondria are typical The cell at *NR* is a nucleated red blood cell as indicated by the greater density of both nucleus and cytoplasm Note the sacs of endo plasmic reticulum The large number of inclusions and the only slightly darker cytoplasm indicate that differentiation has not progressed far Note particularly the well differentiated nucleoplasm with its light and dark areas This is well established early in differentiation in the erythro cytic series The cell at lower right with large nucleus and sparse cyto plasm is not identified

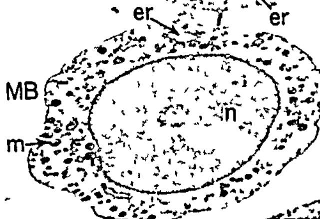
kemia


Bp
m
nl
er
nd
bg


m
MB
n
er



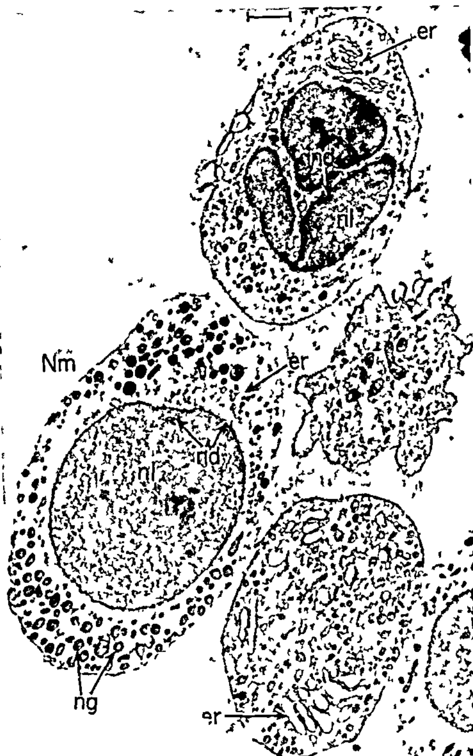

nl
nd
NR
er
er


MB
n
m

(Case 20 before treatment) 13 000 X

Atypical myeloblasts and neutrophilic myelocyte The cell at the upper right has the cytoplasmic characteristics of a myeloblast but the nucleus is well differentiated into light (*nl*) and dark (*nd*) areas with the latter chiefly around the nuclear membrane This is most unusual for myelo blasts which customarily have nucleoplasm of even density The sac like profiles of endoplasmic reticulum (*er*) are characteristic Note their conspicuous appearance in the cell in the lower right corner which has other cytoplasmic characteristics suggesting a myeloblast

The cell at *Nm* is a neutrophilic myelocyte Its distinguishing features are a large number of neutrophilic granules (*ng*) and nucleoplasm that has begun to differentiate into light and dark areas The profiles of endoplasmic recticulum are smaller than is usual for this cell type Cells further advanced in the granulocytic series than myelocytes are seldom seen in myeloblastic leukemia

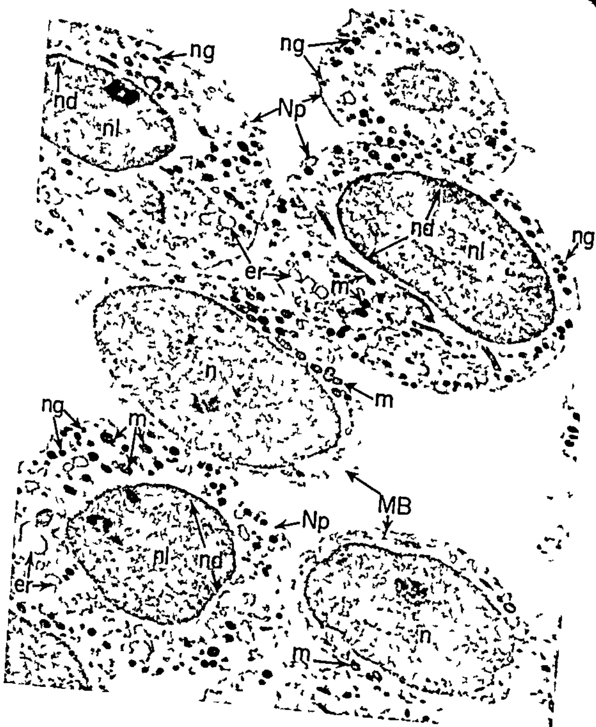

er
nd
nl
Nm
er
nd
nl
ng
er

(Case 20 before treatment) 13 000 X

Atypical myeloblasts and neutrophilic myelocyte The cell at the upper right has the cytoplasmic characteristics of a myeloblast but the nucleus is well differentiated into light (*nl*) and dark (*nd*) areas with the latter chiefly around the nuclear membrane This is most unusual for myeloblasts which customarily have nucleoplasm of even density The sac like profiles of endoplasmic reticulum (*er*) are characteristic Note their conspicuous appearance in the cell in the lower right corner which has other cytoplasmic characteristics suggesting a myeloblast

The cell at *Nm* is a neutrophilic myelocyte Its distinguishing features are a large number of neutrophilic granules (*ng*) and nucleoplasm that has begun to differentiate into light and dark areas The profiles of endoplasmic recticulum are smaller than is usual for this cell type Cells further advanced in the granulocytic series than myelocytes are seldom seen in myeloblastic leukemia

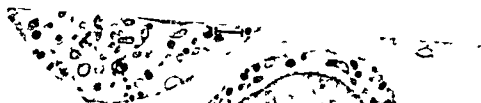
emia


ng
ng
nd
nl
Np
Np
nd
nl
ng
er
m
n
m
ng
m
MB
Np
nl
nd
er
n
m

(Case 20 before treatment) 7500 X

Ambiguous forms This field was chosen to illustrate the difficulty some times encountered in distinguishing myeloblasts from the very early stage in the neutrophilic series the promyelocytes (*Np*) This difficulty arises because myeloblasts have a large number of mitochondria some of which are very small circular in profile and so dense that they often cannot be distinguished from neutrophilic granules

The two cells labeled *MB* are clearly myeloblasts since they possess undifferentiated nucleoplasm (*n*) and mitochondria (*m*) none of which could be mistaken for neutrophilic granules The cells labeled *Np* have nucleoplasm that is in the early stages of differentiation into light (*nl*) and dark (*nd*) areas large profiles of endoplasmic reticulum (*er*) mito chondria (*m*) and what appear to be neutrophilic granules (*ng*) How ever close examination reveals intergrades of size shape and density between mitochondria and granules which leave the identity of many of them uncertain


Nm
nl
nd
ng
er
ng
nl
MB
n
nd
Nmm
nd
nl
ng

(Case 20 before treatment) 7500 X

Nucleoplasmic differentiation These cells illustrate different stages in the differentiation of nucleoplasm This constitutes one of the important criteria in identifying the developmental status of a cell The nucleoplasm (*n*) of the myeloblast (*MB*) is almost entirely undifferentiated having a fairly even density throughout In the cell at *Nm* the nucleoplasm has undergone differentiation into a dark area (*nd*) collected close to the nuclear membrane but the greater part of the interior of the nucleus is of light density (*nl*) The cells at *Nmm* are more advanced the nucleoplasm being clearly divided into light and dark areas The lower one does not appear to be so advanced in its differentiation as the upper one which shows the high contrast between the two densities characteristic of mature neutrophils (pages 36 to 47)

Note that the myelocyte (*Nm*) has large specific granules (*ng*) and large profiles of endoplasmic reticulum (*er*) The metamyelocytes have numerous smaller granules although some large ones are present The endoplasmic reticulum is practically absent

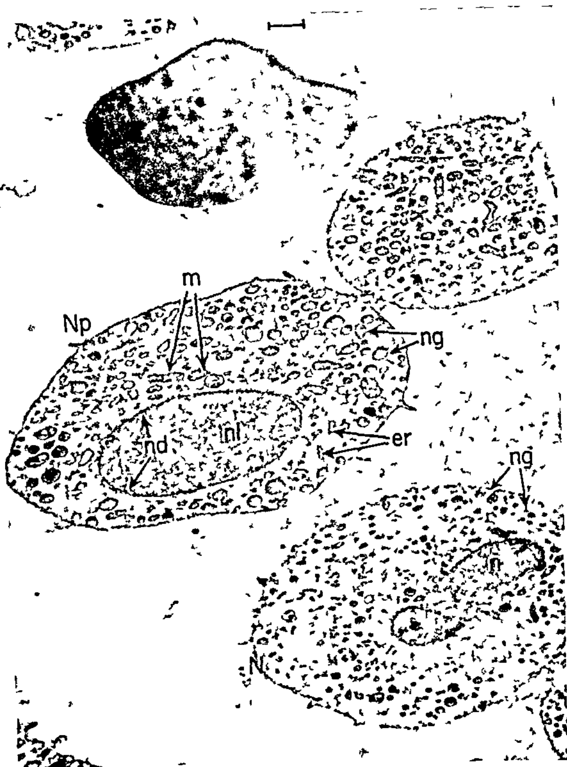
cemia


Nm
nl
nd
er
Np
MB
n
nu
ng

(Case 20 before treatment) 11 000 X

Early and late stages of differentiation At *MB* the even density of the nucleoplasm (*n*) and the two nucleoli (*nu*) indicate an early stage The absence of specific granules suggests a myeloblast The cell at *Np* has a small number of specific granules (*ng*) moderately large profiles of endoplasmic reticulum (*er*) and an undifferentiated nucleus all of which indicate a neutrophilic promyelocyte The cell at *Nm* is further advanced as indicated by the differentiated nucleoplasm (*nl nd*) Al though the neutrophilic granules (*ng*) are not very numerous the nuclear differentiation indicates that this cell has reached the myelocyte stage The cell at the lower margin has the characteristics of a mature monocyte (pages 78 to 85) except that the nucleoplasm is not so well differenti ated as usual

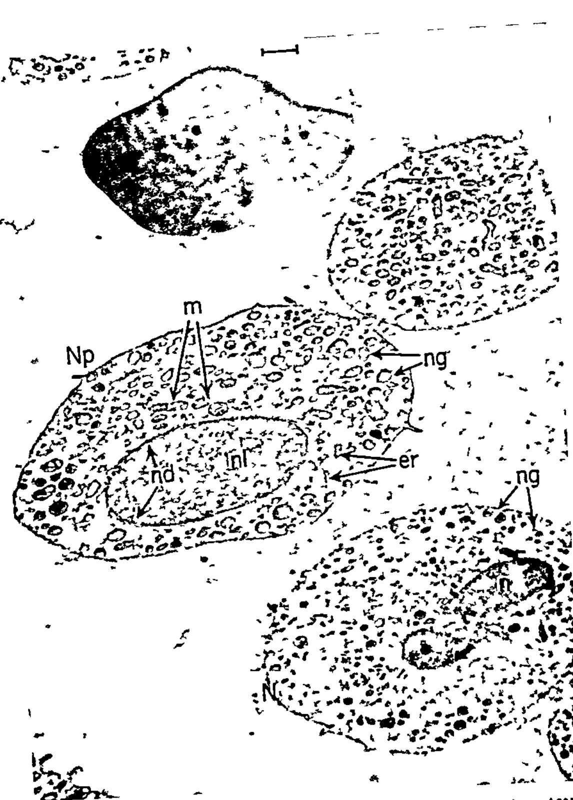
emia


m
Np
ng
nd
nl
er
ng
n
N

(Case 20 before treatment) 9700 X

Neutrophilic forms The cell at *Np* is a neutrophilic promyelocyte the differentiated nucleoplasm (*nl nd*) of which indicates that it may be entering the myelocyte stage The granules (*ng*) although large are interpreted to be neutrophilic The endoplasmic reticulum (*er*) and numerous mitochondria (*m*) are characteristic The mass of cytoplasm above it to the right suggests a cell in the same stage At *N* there is a cell of the neutrophilic series which is apparently further along in its development as indicated by the smaller darker granules (*ng*) and the darker nucleoplasm (*n*)

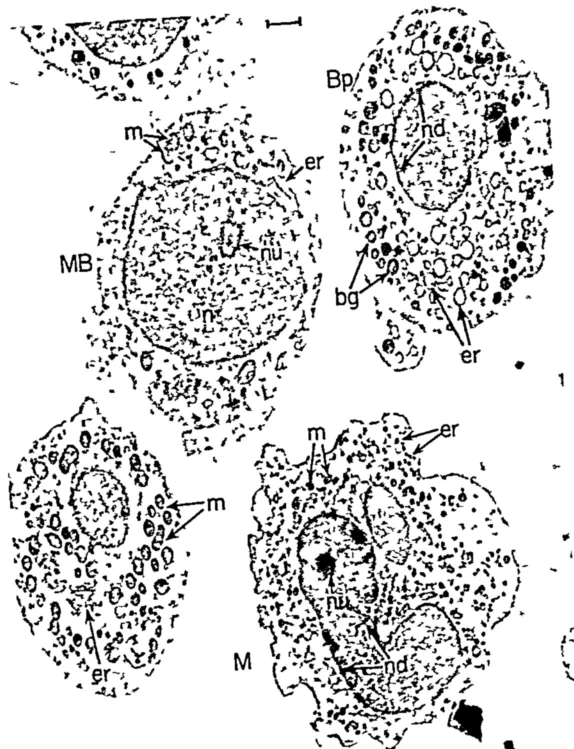
nia


m
Np
ng
er
nd
ng
N

(Case 20 before treatment) 9400 X

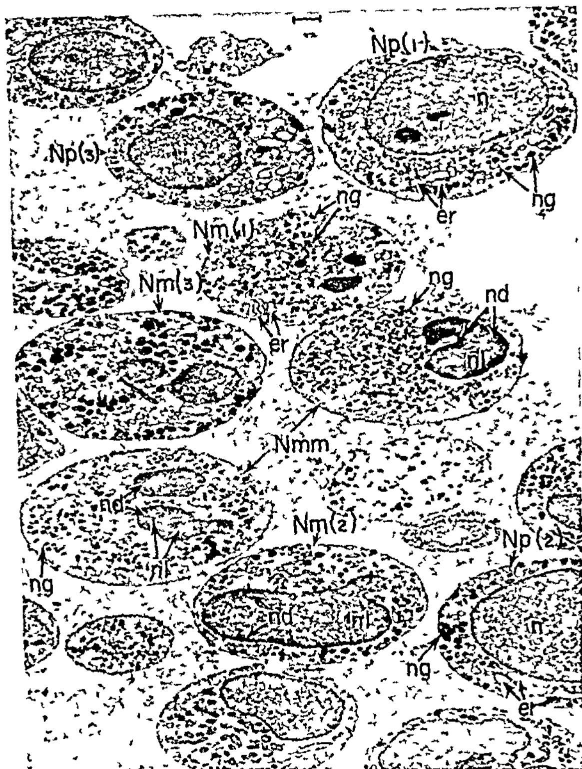
Mixed field The cell at *MB* is a myeloblast with mitochondria (*m*) somewhat less numerous than usual endoplasmic reticulum (*er*) and even density nucleoplasm (*n*) with a nucleolus (*nu*) The cell at *Bp* has large homogeneous granules (*bg*) which suggest that it is a member of the basophilic series The small number of these granules the large circular profiles of endoplasmic reticulum (*er*) and the nucleoplasm barely suggesting differentiation into a dark component (*nd*) near the nuclear membrane indicate that it is still in the promyelocyte stage The cell at *M* has well differentiated nucleoplasm with the dark component around the nuclear membrane small mitochondria and small profiles of endoplasmic reticulum all of which suggest a mature monocyte The only unusual feature of this cell is the nucleolus The cell at lower left has many large mitochondria and abundant small profiles of endoplasmic reticulum the latter feature indicating maturity But there are not enough distinguishing features to permit identification

emia


m
er
MB
nu
n
Bp
nd
bg
er
1
m
er
m
er
M
nu
nd

(Case 20 before treatment) 7100 X

Different stages in granulocytic series Two of the cells *Np*(1) (2) are clearly recognizable as neutrophilic promyelocytes with undifferentiated nucleoplasm (*n*) a small number of specific granules (*ng*) and large profiles of endoplasmic reticulum (*er*) Later stages in the neutrophilic series are represented in the two cells at *Nmm* which are interpreted to be metamyelocytes because of the large number of small specific granules clear differentiation of nucleoplasm into two densities (*nl nd*) and absence of any large profiles of endoplasmic reticulum The irregular nuclear shape suggests banding The cell at *Nm*(1) although nuclear criteria are lacking appears to be in the myelocyte stage This is indicated by the numerous neutrophilic granules (*ng*) many of which are still large and the large profiles of endoplasmic reticulum (*er*) At *Nm*(2) there is another neutrophilic myelocyte which shows nuclear criteria in the form of light and dark areas Although the number of granules is not large the advanced differentiation of the nucleoplasm and the nuclear shape suggest that this cell is approaching the metamyelocyte stage At *Np*(3) and *Nm*(3) there are cells whose granules are somewhat larger than the rest However they are too numerous to be basophilic granules These cells are therefore interpreted to be a neutrophilic promyelocyte at *Np*(3) and a neutrophilic myelocyte at *Nm*(3) Their structural characteristics may be compared to similar cells *Np*(1) (2) and *Nm*(1) (2) elsewhere in the field

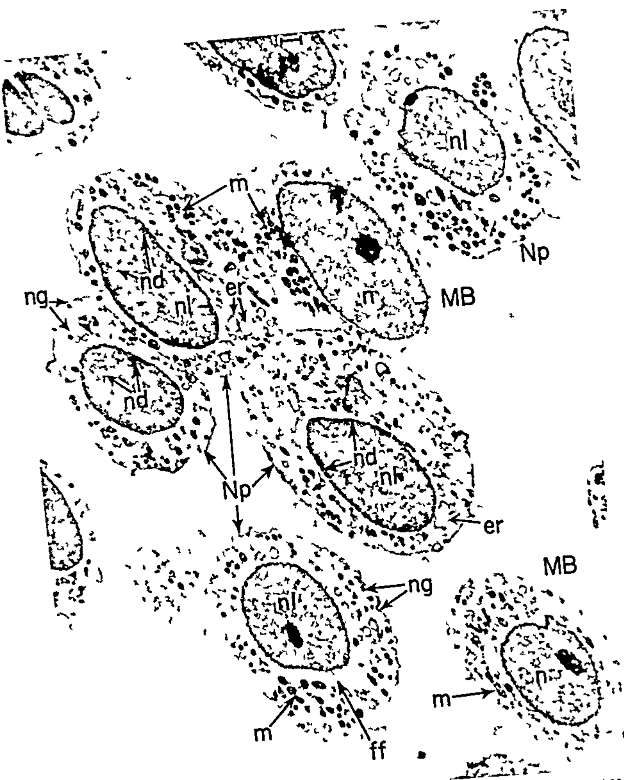


Np(1)
n
ng
er
Np(3)
ng
Nm(1)
Nm(3)
er
ng
nd
nl
Nmm
nd
nl
ng
Nm(2)
Np(2)
nd
nl
n
ng
er

(Case 20 before treatment) 7100 X

Different stages in granulocytic series Two of the cells *Np*(1) (2) are clearly recognizable as neutrophilic promyelocytes with undifferenti ated nucleoplasm (*n*) a small number of specific granules (*ng*) and large profiles of endoplasmic reticulum (*er*) Later stages in the neutro philic series are represented in the two cells at *Nmm* which are inter preted to be metamyelocytes because of the large number of small spe cific granules clear differentiation of nucleoplasm into two densities (*nl nd*) and absence of any large profiles of endoplasmic reticulum The irregular nuclear shape suggests banding The cell at *Nm*(1) although nuclear criteria are lacking appears to be in the myelocyte stage This is indicated by the numerous neutrophilic granules (*ng*) many of which are still large and the large profiles of endoplasmic reticulum (*er*) At *Nm*(2) there is another neutrophilic myelocyte which shows nuclear criteria in the form of light and dark areas Although the number of granules is not large the advanced differentiation of the nucleoplasm and the nuclear shape suggest that this cell is approaching the metamyelo cyte stage At *Np*(3) and *Nm*(3) there are cells whose granules are somewhat larger than the rest However they are too numerous to be basophilic granules These cells are therefore interpreted to be a neutro philic promyelocyte at *Np*(3) and a neutrophilic myelocyte at *Nm*(3) Their structural characteristics may be compared to similar cells *Np*(1) (2) and *Nm*(1) (2) elsewhere in the field

emia

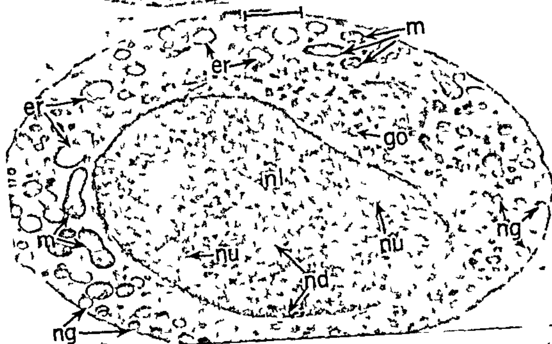

nl
m
Np
nd
nl
er
MB
m
ng
nd
nd
nl
Np
er
MB
ng
nl
n
m
m
ff

(Case 20 before treatment) 5300 X

Neutrophilic series and myeloblasts (MB) Both the myeloblasts have undifferentiated nucleoplasm (*n*) without light or dark areas Small mito chondria (*m*) are present but in the cell at lower right they are some what less numerous than usual An unusual feature of these two cells is the almost total absence of endoplasmic reticulum which is usually abundant at this early stage The remainder of the cells are neutrophilic promyelocytes (*Np*) They are typical in that the nucleoplasm has only begun to differentiate into light (*nl*) and dark areas (*nd*) the latter being present chiefly just inside of the nuclear membrane Other features indi cating an early stage of development are the small number of neutro-philic granules (*ng*) the fairly numerous mitochondria (*m*) and the abundant large profiles of endoplasmic reticulum (*er*) mostly circular *or oval The cell at lower center has a distinct fibrillar formation (fl)* (pages 309 and 311)

kemia

ng
m
ng
er
nd
nl
nd
m
er



m
er
er
go
nl
nu
nu
ng
m
nd
ng

Neutrophilic promyelocytes

Upper (Case 20 before treatment) **15 000 X**
The specific granules (*ng*) are sparse and scattered randomly and tend to be large The endoplasmic reticulum (*er*) is present in large profiles Many of them tend to have a cisternal shape which is perhaps an indication that this cell has not progressed far from the myeloblast stage in which cisternal profiles are common Mitochondria (*m*) are evident The nucleoplasm is not well differentiated there being only slight indication of light (*nl*) and dark (*nd*) areas

Lower (Case 20 before treatment) **15 000 X**
The specific granules (*ng*) are few and randomly distributed The endoplasmic reticulum (*er*) is present in large profiles which tend to be round or oval and there are numerous mitochondria (*m*) There is a Golgi zone (*go*) The nucleoplasm has undergone some differentiation into light (*nl*) and dark (*nd*) areas and two nucleoli (*nu*) are present



STEM CELL LEUKEMIA

The name *stem cell* implies a forerunner of more specific cell types It is the same cell type that has been called a myeloblast (hemocytoblast) in the section on myeloblastic leukemia Stem cell leukemia is presented in a separate section because of the presence of certain cells which have not been observed elsewhere in the course of this study The first two micrographs illustrate a typical field in an untreated case of this leukemia The electron morphology of these stem cells is that of typical myeloblasts and they are therefore so labeled in the illustrations Myeloblasts have been described in more detail elsewhere (pages 198 to 231) This is the characteristic picture before treatment The remaining micrographs are from a patient who had been treated with 40 mg 6-mercaptopurine every 2 days for 4 days The predominating cell type the stem cell responsible for the diagnosis possesses mature characteristics and does not correspond to any other cell type encountered in this study One field is presented from this case (page 243) which suggests a transition from myeloblast to this supposed stem cell in a series of five progressive forms True myeloblasts are rare in this specimen but one field (page 245) illustrates both myeloblast and stem cell The remainder of the section deals with the morphology of this extremely ambiguous cell type which according to criteria now available is a structurally mature stem cell

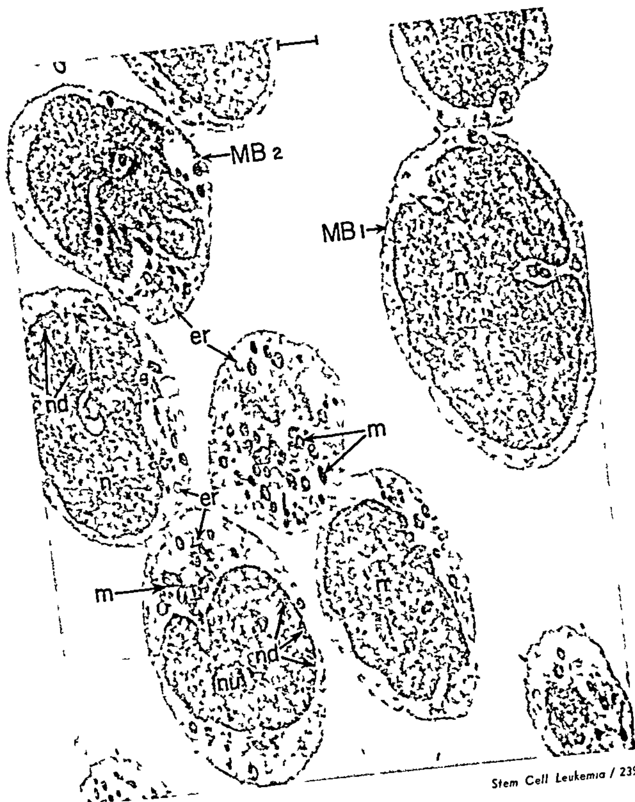
STEM CELL LEUKEMIA MICROGRAPHS

STEM CELL LEUKEMIA MICROGRAPHS

(Case 21) 11 000 X

Primitive cell type These cells are essentially undistinguishable from the myeloblasts of myeloblastic leukemia (pages 198 to 231) The nucleoplasm (*n*) is largely undifferentiated but there is suggestion of an approach to differentiation in the form of scattered areas of dark nucleoplasm (*nd*) located chiefly along the nuclear membrane Nucleoli (*nu*) are present The nuclei of these cells are generally large and of fairly even contour (*MB1*) but they may be quite irregular (*MB2*) Mitochondria (*m*) are numerous in the cytoplasm and some endoplasmic reticulum (*er*) is present The profiles of the latter tend to be small and not so numerous as those of actively differentiating cells in the granulocytic series

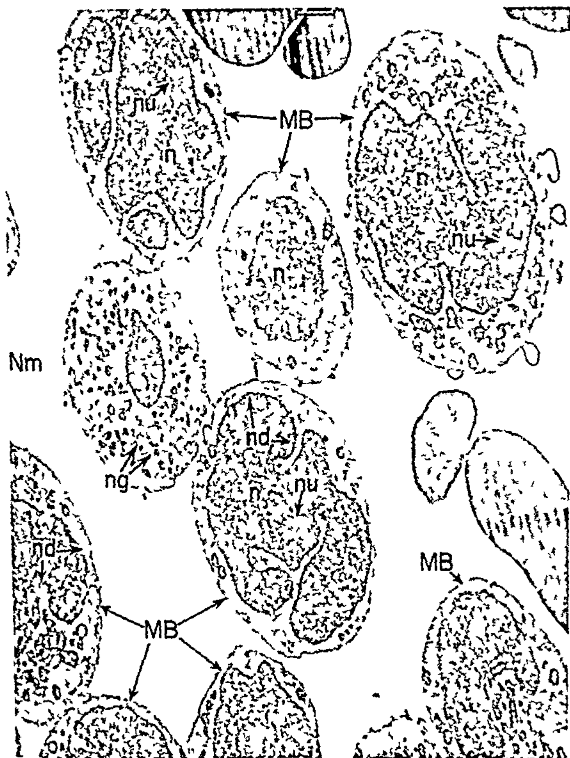
nia


MB 2
MB 1
er
nd
n
m
er
m
nd
nu

(Case 21) 10 000 X

Primitive cell type and neutrophilic myelocyte The predominant cell type in this stem cell leukemia which had not been treated at the time the blood sample was taken is labeled *MB* in this micrograph This notation was chosen because these cells are essentially undistinguishable from the myeloblasts of myeloblastic leukemia (pages 198 to 231) The nuclei are largely undifferentiated (*n*) but there is a slight tendency for certain areas of the nucleoplasm to be darker (*nd*) chiefly around the nuclear membrane Nucleoli (*nu*) are present The cytoplasm contains numerous mitochondria but no other distinguishing features of note The cell at *Nm* is a member of the neutrophilic series with specific granules (*ng*) and is probably about midway along in its development

mia


MB
nu
n
n
nu
Nm
nd
nu
n
ng
nd
MB
MB

(Case 22) 9900 X

Cells suggesting transition from myeloblasts to stem cells The cell at *MB* shows very little nucleoplasmic differentiation (*n*) the surest sign of an early stage in any cell series It has the same characteristics as the myeloblasts of myeloblastic leukemia by the standards of light mi croscopy There are four other cells in the field which have the char acteristics of stem cells whose nucleoplasm seems to represent a progressive differentiation into light (*nl*) and dark (*nd*) areas The cell at S_1 appears to be in an early stage of nucleoplasmic differentiation but already has the general appearance of a stem cell However it is not far removed in appearance from the myeloblast (*MB*) The cells labeled S_2 S_3 and S_4 are interpreted to represent further progressive stages leading to the mature stem cell type illustrated on subsequent pages S_4 being at or close to the typical appearance (pages 245 to 253 257 and 259)

The cell at *U* is an unknown form that merits special attention be cause of its peculiar structural features and the fact that it represents a type fairly frequently observed in these preparations Note that the dark area of the differentiated nucleoplasm completely surrounds a light area This sometimes occurs with very little accompanying cytoplasm to such an extent that practically only the nucleus is visible This results in a centrally located light area of nucleoplasm that could be mistaken for cytoplasm In this cell the mitochondria (*m*) are swollen and have clear matrices

Elsewhere in the field are neutrophilic forms One appears to be a *myelocyte (Nm) and th~ other two are metamyelocytes (Nmm)*


S4
nl
nd
S1
n
MB
U
m
nd
S3
Nmm
S2
nl
nl
nd
m
Nm

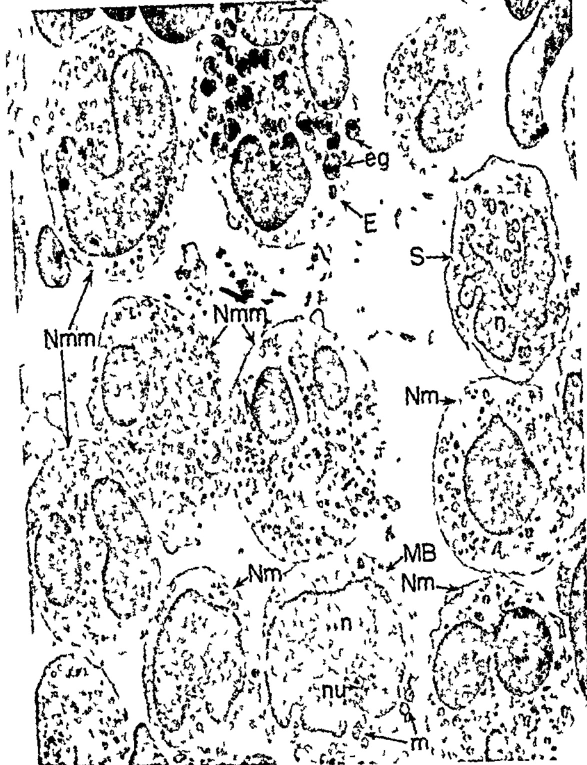
(Case 22) 9900 X

Cells suggesting transition from myeloblasts to stem cells The cell at *MB* shows very little nucleoplasmic differentiation (*n*) the surest sign of an early stage in any cell series It has the same characteristics as the myeloblasts of myeloblastic leukemia by the standards of light mi croscopy There are four other cells in the field which have the char acteristics of stem cells" whose nucleoplasm seems to represent a progressive differentiation into light (*nl*) and dark (*nd*) areas The cell at *S1* appears to be in an early stage of nucleoplasmic differentiation but already has the general appearance of a stem cell However it is not far removed in appearance from the myeloblast (*MB*) The cells labeled *S_* *S3* and *S4* are interpreted to represent further progressive stages leading to the mature stem cell type illustrated on subsequent pages *S4* being at or close to the typical appearance (pages 245 to 253 257 and 259)

The cell at *U* is an unknown form that merits special attention be cause of its peculiar structural features and the fact that it represents a type fairly frequently observed in these preparations Note that the dark area of the differentiated nucleoplasm completely surrounds a light area This sometimes occurs with very little accompanying cytoplasm to such in extent that practically only the nucleus is visible This results in a centrally located light area of nucleoplasm that could be mistaken for cytoplasm In this cell the mitochondria (*m*) are swollen and have clear matrices

Elsewhere in the field are neutrophilic forms One appears to be a myelocyte (*Nm*) and the other two are metamyelocytes (*Nmm*)

mia



eg
E
S
n
Nmm
Nmm
Nm
MB
Nm
Nm
n
nu
m

(Case 22) 9300 X

Myeloblasts and stem cells At the bottom of this field there is a cell (*MB*) which has the same appearance as the myeloblasts observed in myeloblastic leukemia (pages 198 to 231) Note that the nucleoplasm (*n*) is largely undifferentiated except for the nucleolus (*nu*) The cyto plasm is moderately dense and contains numerous mitochondria (*m*) The cell at *S* has the typical stem cell appearance except that the nucleoplasm is not so conspicuously divided into light and dark areas as in many stem cells It should be compared with the myeloblast (*MB*) There is nothing about the cell at *E* that serves to distinguish it from a mature eosinophil Note however the messy and disorganized appearance of the specific granules (*eg*) a typical situation in this par ticular case which may have resulted from the treatment The neutro philic forms present are either myelocytes (*Nm*) or metamyelocytes (*Nmm*) and show no unusual features (see pages 166 to 173 for criteria)

ıa

I
Nmm
S
nl
R
S
R
T
S
L
NR
R
R
nl
nl
m
S
S
N
nd
fb
S
S
Emm
T
Stem Cell Leuk

(Case 22) 6500 X

Multiple cell types A wide variety of cells are found in this particular specimen The predominating type is the stem cell (S) with its nucleus often of irregular contour containing nucleoplasm well differentiated into light (nl) and dark (nd) areas The cytoplasm contains conspicuous although not particularly numerous mitochondria (m) which frequently have lucid matrices The undifferentiated cytoplasm is finely speckled and rather dense Note how the contrast between the nucleoplasmic densities differs from cell to cell

A *mature lymphocyte* (L) *is present and contrasts markedly with the* nucleated erythrocyte (NR) both in nuclear pattern and in cytoplasmic density Note that the density of the cytoplasm of the stem cells is between the extremes represented in the lymphocyte and the nucleated red blood cell Several members of the neutrophilic series can be recognized Those at *Nmm* are metamyelocytes and that at N is apparently a mature neutrophil Also present are blood platelets (T) erythrocytes (R) and fibrin (fb)

This field is from a nearby section of the same specimen close to the field on page 249 Certain of these cells including the mature lymphocyte (L) appear in both fields This helps identify the cell labeled *Emm* as an eosinophilic metamyelocyte

mia


L
S
nP
nd
eg
S
nl
nd
m
Emm
S
nd
nd
nl
fb
T
R
R
NR
nl
nd
er
S

(Case 22) 6500 X

Mixed field This low power field contains a large number of cell types The cells labeled *S* are unique to this type of leukemia and despite certain contradictory features of ultrastructure described elsewhere (pages 256 to 259) are designated stem cells In general they are characterized by nuclei of rather irregular contour with nucleoplasm that is clearly differentiated into light (*nl*) and dark (*nd*) areas The most conspicuous feature of the cytoplasm is the mitochondria (*m*) which have very dense cristae and nearly transparent matrices The cytoplasm is more or less speckled usually without conspicuous endo plasmic reticulum However some cells of this type may have large profiles of endoplasmic reticulum (*er*) as in the cell in the lower right corner

Other cells present include an eosinophilic metamyelocyte (*Emm*) which is typical except for the disorganized appearance of the specific granules (*eg*) A mature lymphocyte at *L* has the characteristically lucid cytoplasm which distinguishes the lymphocytic series from other cells The cell at *NR* is an early stage in the erythrocytic series The cytoplasm is of more even density and somewhat darker than that of other cells and the nucleoplasm at this stage has distinctly differentiated light and dark areas Although superficially resembling the stem cells it can be distinguished by the more even density of its cytoplasm the regular con tour of its nucleus and the distinctly different pattern of differentiation

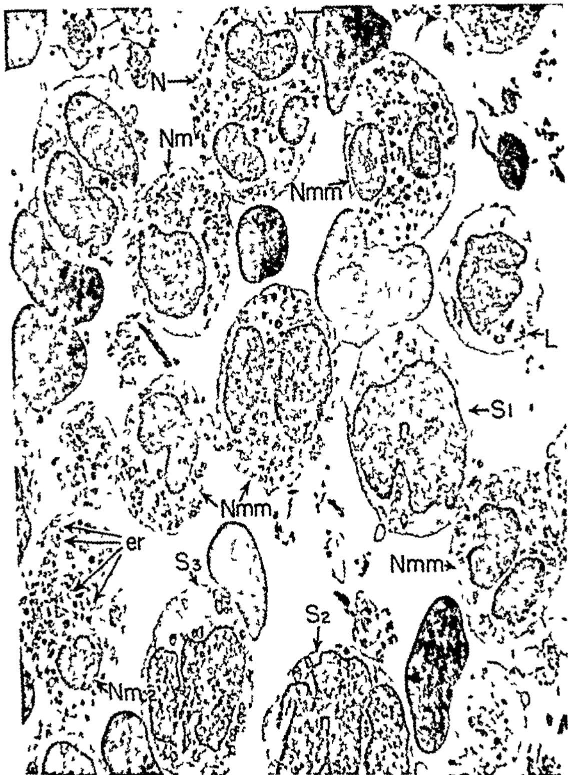
Also present in this field are erythrocytes (*R*) blood platelets (*T*) and fibrin (*fb*)

ia


L
S
nP
nd
eg
S
nl
nd
m
Emm
S
nd
nl
fb
R
R
NR
nl
nd
er
S

(Case 22) 7500 X

Stem cell variations This field illustrates a number of stem cells (S) and neutrophilic forms (Nm Nmm N) all of which have developed to the myelocyte stage or beyond The stem cells are typical but the one at S_1 appears to be about midway along in the differentiation of its nucleoplasm (n) Those at S_2 and S_3 have nucleoplasm that approaches more nearly the usual appearance The neutrophilic forms have no unusual features that at N apparently being a mature neutrophil The metamyelocytes (Nmm) show signs of banded nuclei and the myelocytes have either rounded nuclei (Nm_1) or large profiles of endoplasmic reticulum (er) (Nm_2) The cell at L appears to be a mature lympho cyte although the nucleoplasmic contrast is more clearly established than usual (see pages 64 to 77)


N
Nm
Nmm
L
S1
Nmm1
er
S3
Nmm
S2
Nm2

(Case 22) 10 000 X

Stem cells and members of neutrophilic series The cells labeled *S* are typical of their kind Characteristic features are the well differentiated nucleoplasm with conspicuous light (*nl*) and dark (*nd*) areas and finely speckled cytoplasm of moderate density with conspicuous mitochondria (*m*) Nuclear contour may be quite irregular

The cell at *Np* has largely undifferentiated nucleoplasm (*n*) numerous mitochondria (*m*) large profiles of endoplasmic reticulum (*er*) and two small areas of granules (*ng*) which appear to be neutrophilic All these characteristics suggest a neutrophilic promyelocyte At *Nm* a some what later stage is represented in which the nucleoplasm is differentiated into light and dark areas Although the number of neutrophilic granules (*ng*) may not represent the full complement they are numerous enough to indicate that the myelocyte stage has been reached a conclusion sup ported by the nucleoplasmic differentiation The remaining members of the neutrophilic series (*Nmm*) are metamyelocytes Note the advanced nucleoplasmic differentiation the large number of granules many of which are small and the nuclear contours suggesting banding The endoplasmic reticulum is also reduced


m
nd
S
nl
Nmm
ng
nd
nl
nd
nl
m
nl
nd
Nm
R
S
m
er
ng
nd
nl
R
ng
n
Np

(Case 22) 10 000 X

Early developmental forms Although no stem cells are present in this field it serves to illustrate some of the immature forms encountered in this treated case The cell at *Bm* is a member of the basophilic series as indicated by the lack of ultrastructure within the granules (*bg*) Nucleoplasmic differentiation has progressed to recognizable light (*nl*) and dark (*nd*) areas and there are numerous large profiles of endoplasmic reticulum (*er*) This cell is entering the myelocyte stage At *Emm* is an eosinophilic metamyelocyte with typical structural features The granules (*eg*) appear to be in poor condition a common finding in this particular case The neutrophilic forms are found in the myelocyte (*Nm*) and metamyelocyte (*Nmm*) stages (for criteria see pages 166 to 173)

mia


Nm
Nmm
er
nd
nl
bg
Bm
Emm
Nm
eg
Nmm

(Case 22) 10 000 X

Early developmental forms Although no stem cells are present in this field it serves to illustrate some of the immature forms encountered in this treated case The cell at *Bm* is a member of the basophilic series as indicated by the lack of ultrastructure within the granules (*bg*) Nucleoplasmic differentiation has progressed to recognizable light (*nl*) and dark (*nd*) areas and there are numerous large profiles of endo plasmic reticulum (*er*) This cell is entering the myelocyte stage At *Emm* is an eosinophilic metamyelocyte with typical structural features The granules (*eg*) appear to be in poor condition a common finding in this particular case The neutrophilic forms are found in the myelocyte (*Nm*) and metamyelocyte (*Nmm*) stages (for criteria see pages 166 to 173)

a


m
nd
nl
er
nd
nl
er

(Case 22) 16 000 X

Stem cells The cytoplasm is of medium density Endoplasmic reticulum (*er*) is sparse and where present is found chiefly in the form of profiles of flattened sacs Mitochondria (*m*) are fairly numerous and mostly round but rod shaped forms exist The nucleoplasm shows a high degree *of differentiation into light (nl) and dark (nd) areas In general the* dark component forms an irregular and often incomplete band around the nucleus inside the nuclear membrane with irregular and apparently isolated clumps located anywhere in the nucleus

Although the stem cell is generally regarded as an early and very immature form capable of giving rise to mature cells through the granu *locytic and erythroblastic series practically all the structural features* of these cells are characteristic of maturity The only other cell type possessing comparable nucleoplasmic differentiation before final maturity is the erythroblast (see pages 338 to 343) The cytoplasm of these cells although a less reliable indicator of maturity does not possess the copious endoplasmic reticulum which is so conspicuous in many early forms *For other puzzling features of this cell type see page 259*

nia


m
nd
nl
er
nd
er
nl

Stem cells Both these fields illustrate typical cells identified as stem cells by the criteria of light microscopy in this clinically treated stem cell leukemia

Upper (Case 22) 23 000 X

The rather dense cytoplasm contains conspicuous mitochondria (*m*) which are well rounded and sparse endoplasmic reticulum (*er*) in the form of flattened sacs The nucleoplasm shows a high degree of differentiation into light (*nl*) and dark (*nd*) areas The dark component is well scattered throughout the nucleus as well as forming an irregular band inside the nuclear membrane

Although supposedly a primitive cell type identical with the myeloblast these cells have characteristically mature ultrastructure and do not at all resemble the myeloblasts of the myeloblastic leukemias (see pages 198 to 231) which are typical undifferentiated cells

Lower (Case 22) 18 000 X

This stem cell has the usual moderately dense cytoplasm sparse saclike endoplasmic reticulum (*er*) and round or oval mitochondria (*m*) The irregular nuclear contour and advanced nucleoplasmic differentiation (*nl nd*) are both typical Although this cell type has mature structural features it is different from any mature leukocytes of normal blood (see pages 15 to 87) or indeed from any cell type observed in any of the leukemias described in this volume

a


m
er
nl
nd
er
m
er
nd
nl

Stem cells Both these fields illustrate typical cells identified as stem cells by the criteria of light microscopy in this clinically treated stem cell leukemia

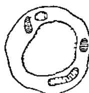
Upper (Case 22) 23 000 X

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Although supposedly a primitive cell type identical with the myeloblast these cells have characteristically mature ultrastructure and do not at all resemble the myeloblasts of the myeloblastic leukemias (see pages 198 to 231) which are typical undifferentiated cells

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This stem cell has the usual moderately dense cytoplasm sparse saclike endoplasmic reticulum (*er*) and round or oval mitochondria (*m*) The irregular nuclear contour and advanced nucleoplasmic differentiation (*nl nd*) are both typical Although this cell type has mature structural features it is different from any mature leukocytes of normal blood (see pages 15 to 87) or indeed from any cell type observed in any of the leukemias described in this volume



LYMPHOCYTIC LEUKEMIA

The specimens of buffy coat from lymphocytic leukemia examined in this study have a high proportion of immature forms recognizable by the undifferentiated nature of the nucleoplasm and a generous amount of cytoplasm of very low density Nucleoplasmic differentiation into dark and light areas present in a few of the cells is the chief indication of progress toward maturity Reduction of cytoplasm is an added indication but is not particularly reliable since mature forms often contain considerable cytoplasm

In one case of subacute lymphocytic leukemia multilobed nuclei were present Examples of these cells are illustrated on pages 277 and 279 The extremely irregular nuclear contours are emphasized by the thinness of the sections

A brief account of sectioned cells in lymphocytic leukemia has been presented by Bernhard and Leplus [1] The present status of lymphocytic leukemia has been reviewed by Bessis [2 chap XII]

REFERENCES

1 Bernhard W and R Leplus Perspectives nouvelles en cytologie sanguine Institut de Recherches sur le Cancer Gustave Roussy Villejuif

2 Bessis M Cytology of the blood and blood forming organs Grune and Stratton New York 1956

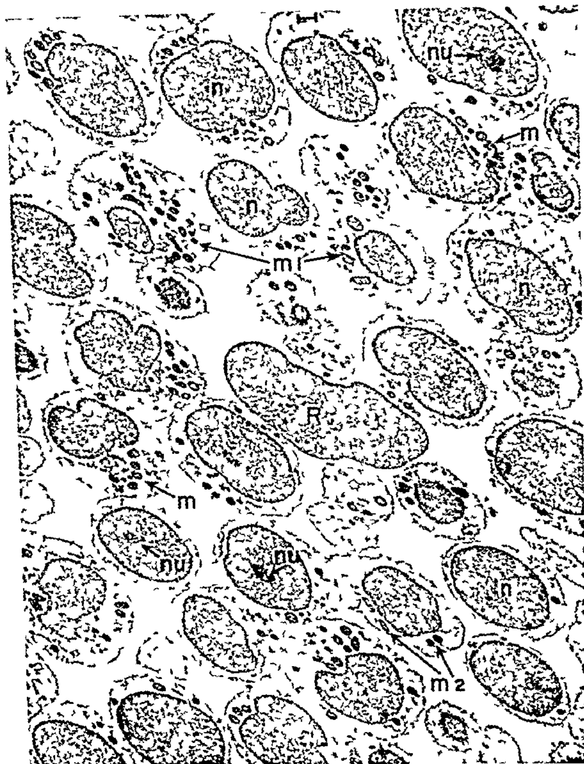
LYMPHOCYTIC LEUKEMIA MICROGRAPHS


nu
nu
R
n
m
m
n
m
n
R
nu

(Case 26 after treatment) 1100 X
Smear of buffy coat A few mature lymphocytes (*L*) are present, but the remainder of the cells are immature lymphocytic forms

(Case 26 after treatment) 4900 X
Developing lymphocytic forms This is a typical low power field With the exception of occasional erythrocytes (*R*) the cells are all members of the lymphocytic series These cells have large usually well rounded nuclei with nucleoplasm (*n*) of even density There are occasional nucleoli (*nu*) which are of only slightly greater density than the surrounding nucleoplasm The cytoplasm is sparse and very light with numerous mitochondria (*m*) Higher magnification is required for the demonstration of finer cytoplasmic detail (pages 273 to 279)

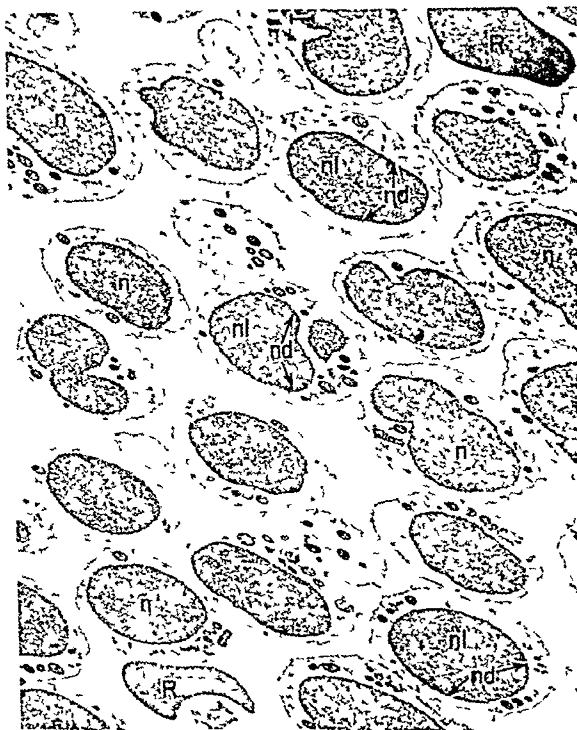
mia


nu
m
n
n
mi
n
R
m
nu
nu
n
m2

(Case 26 after treatment) 4900 X

Cells of lymphoblastic series The well rounded nuclei with occasional nucleoli (nu) have a typically even density of nucleoplasm (n) with little suggestion of differentiation into a mature nuclear pattern A very conspicuous and constant feature of these cells is the very light undifferentiated cytoplasm which contrasts with the high density of the contained mitochondria (m) The size and density of the mitochondria may vary considerably from cell to cell (m_1) or even in the same cell (m_2) Other cytoplasmic structures are too small for identification at this low level of magnification An erythrocyte (R) is also present

emia


R
n
nl
nd
n
nl
nd
n
n
n
n
nL
nd
R

(Case 26 after treatment) 6100 X

Immature lymphocytes There is a conspicuous lack of differentiation of nucleoplasm (*n*) In mature lymphocytes of normal blood there are light and dark areas of nucleoplasm (pages 64 to 77) In the cells here illustrated there is only a faint suggestion of nucleoplasmic differentiation into light (*nl*) and dark (*nd*) areas In most other leukocytic forms this is an indication of immaturity and it probably is here also However it is difficult to judge their stage of development since the mature condition does not present strong contrasts or coherent organizational patterns Some erythrocytes (*R*) are present

mia



R
n
nl
nd
n
nl
nd
n
n
nl
nd
R

(Case 26 after treatment) 9800 X

Lymphoblasts The cells in this field are typical of those that make up the extremely large buffy coat in the lymphocytic leukemias The nuclei are large and well rounded and possess nucleoplasm of even density (*n*) and occasional nucleoli (*nu*) The generally clear and very light cyto plasm contains numerous mitochondria (*m*) Endoplasmic reticulum (*er*) is present but not conspicuous both as large profiles and as aggregates of extremely small ones Since these cells do not possess the morphologic characteristics of the mature lymphocytes of normal blood they must be interpreted as lymphoblasts Two of their structural features support this interpretation the undifferentiated nucleoplasm and the occasional large profiles of endoplasmic reticulum

kemia


n
n
er
er
nu
n
nu
m
er
n
n
er

(Case 26 after treatment) 9800 X

Typical lymphoblasts The most notable feature is the undifferentiated nucleoplasm (*n*) which is of even density throughout A second indication of immaturity is the common occurrence of large circular or oval profiles of endoplasmic reticulum (*er*) Other notable features of these cells are the nucleoli (*nu*) and the conspicuous mitochondria (*m*) The cytoplasm except for the mitochondria and endoplasmic reticulum is unusually clear It is the most lucid cytoplasm found in any of the leukocytic forms mature or otherwise Some erythrocytes (*R*) are present

mia


R
n
nu
n
er
nu
er
m
R
n
er
n
nu
er
m

(Case 26 after treatment) 9800 X

Typical lymphoblasts The most notable feature is the undifferentiated nucleoplasm (n) which is of even density throughout A second indication of immaturity is the common occurrence of large circular or oval profiles of endoplasmic reticulum (er) Other notable features of these cells are the nucleoli (nu) and the conspicuous mitochondria (m) The cytoplasm except for the mitochondria and endoplasmic reticulum is unusually clear It is the most lucid cytoplasm found in any of the leukocytic forms mature or otherwise Some erythrocytes (R) are present

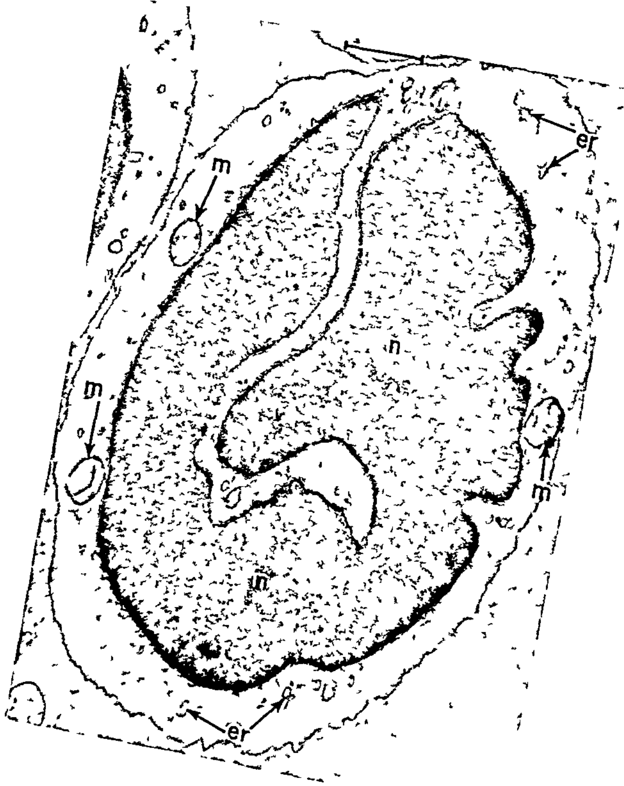
mia


f
er
n
n
m
n
n
er
n
er

(Case 26 after treatment) 17 000 X

Atypical ultrastructure These cells are typical lymphoblasts which are somewhat more compressed through centrifugation or sectioning than is usual Their nuclei show the even density of nucleoplasm (*n*) Mitochondria (*m*) and profiles of endoplasmic reticulum (*er*) are scattered in very light cytoplasm Occasionally a fibrillar appearance (*f*) is observed in the cytoplasm of these cells Since it is not a constant feature of their ultrastructure it may be a fixation artifact

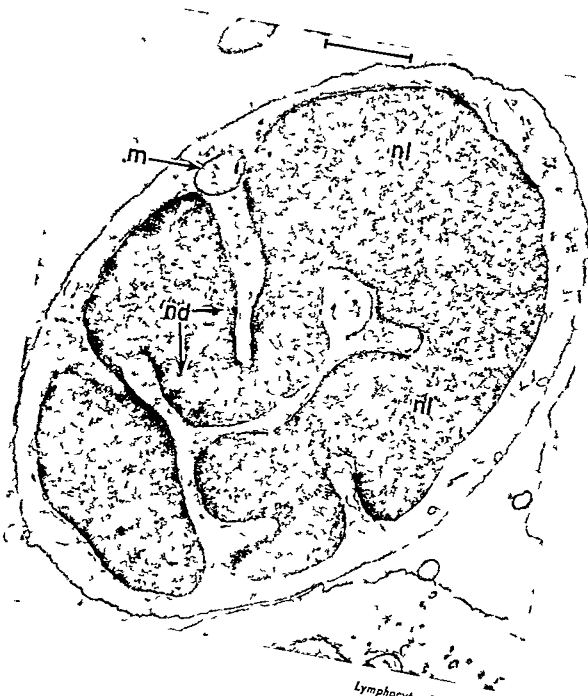
emia


m
er
m
n
m
n
er

(Case 30 before treatment) 21 000 X

Immature lymphocyte In some cases of lymphocytic leukemia cell forms are observed which have nuclei of very complex contours that are re ferred to as *multilobed* The extreme convolutions of the nucleus in this field are characteristic of this type of cell The even density of the nucleoplasm (*n*) indicates an immature form Mitochondria (*m*) and endoplasmic reticulum (*er*) are present The cytoplasm is light and generally clear as is characteristic of lymphocytes

ıkemia

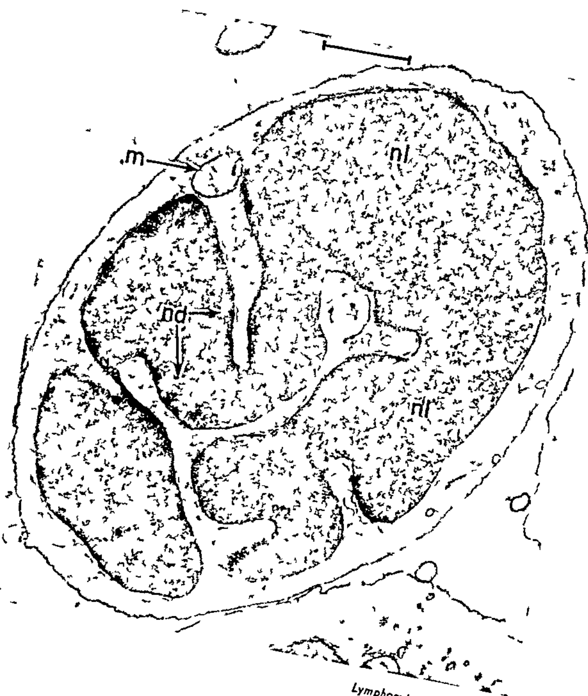

m
nl
nd
nl

(Case 30 before treatment) 23 000 X

Lymphocyte This cell is typical of the multilobed cells that occasionally occur in the lymphocytic leukemias The extremely convoluted nucleus is emphasized by the thin section The differentiation of the nucleus into light (*nl*) and dark (*nd*) areas and the sparse cytoplasm both indicate maturity

The unusually clear areas in the matrix of the mitochondrion (*m*) are not uncommon in these preparations

1a


m
nl
nd
nl



MONOBLASTIC LEUKEMIA

The monoblastic leukemias serve to illustrate the developmental stages in the monocytic series The forms here illustrated represent chiefly the early blastic forms No chronic form such as might possess mature cells is known to exist

There is often an aberration of the plasma components in the leukemias This is probably responsible for the precipitated protein in most of the low power micrographs of this section However in keeping with a policy of minimal operations on the blood samples both before and after fixation no attempt was made to eliminate it It does not appear to have affected the quality of the preparations except by giving a mottled appearance to the background

The monoblasts which prevail in these preparations are readily recognizable as immature forms by the undifferentiated nature of their nucleoplasm The cytoplasm is uniformly darker and the nuclei uniformly lighter than the corresponding areas in the occasional lymphocytes found in some of the fields Otherwise monoblasts have no particular characteristics that might distinguish them from other cells of the agranulocytic group

Auer bodies which are sometimes found in acute leukemias are present in these preparations and are described on pages 290 304 and 306 Their external form may vary considerably but the trend is toward

a rod shaped structure circular in cross section However dumbbell shaped Auer bodies have been observed (page 305) There is faint suggestion of internal ultrastructure in some of them in the form of granules and vaguely linear areas but there seems to be no uniformity Their internal make up represents at best a poor level of structural organization

Certain structures found in monoblasts in these cases and described by Bessis and Breton Gorius [2] as crescentic areas have been illus trated (pages 299 303 309 311) Since haphazard plane of section can change the apparent shape of any formed element except a sphere a name implying a certain shape is an unfortunate descriptive choice It was therefore considered advisable to refer to these structures as *fibrillar formations* since they appear to be essentially aggregates of fine fibers It is particularly interesting that the area of cytoplasm enclosed by them has a distinctly different texture from that of the remaining cytoplasm of the cell These fibrillar formations have been observed in other cell types (pages 151 165 211 231) in the course of this study

The monoblastic leukemias have received little attention in electron microscopy The present status of our knowledge has been reviewed by Bessis [1 chap XIII]

REFERENCES

1 Bessis M Cytology of the blood and blood forming organs Grune and Strat ton New York 1956
2 Bessis M and J Breton Gorius Examen au microscope electronique des cellules des leucemies myeloides *Bull Micr Appl* 5 9–11 1955

MONOBLASTIC LEUKEMIA MICROGRAPHS

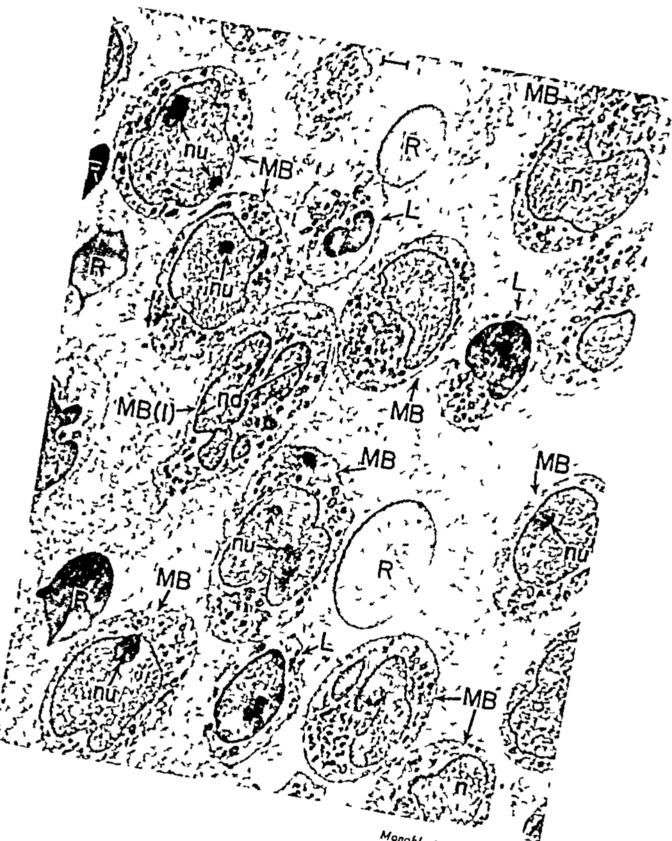
(Case 34a before treatment) 6900 X

Blast forms of monocytic series This micrograph presents a typical low power field from the buffy coat in a monoblastic leukemia The granular appearance of the extracellular background is due to precipitated protein It may be traceable to an excess of protein in the blood plasma The general appearance of this precipitate is that of a blood clot without fibrin

Most of the cells in this field are monoblasts (*MB*) These have nucleoplasm (*n*) of fairly even density except for prominent and very dense nucleoli (*nu*) These cells may be distinguished from mature monocytes by the fact that there is little or no tendency for dark nucleoplasm (*nd*) to collect subjacent to the nuclear membrane (pages 78 to 85) The cell labeled *MB*(*1*) is nearly mature It is difficult to distinguish it from a mature monocyte

This micrograph also contains erythrocytes (*R*) Three lymphocytes (*L*) are recognizable by their light cytoplasm More detailed criteria for distinguishing lymphocytes from monoblasts are given on pages 292 and 293

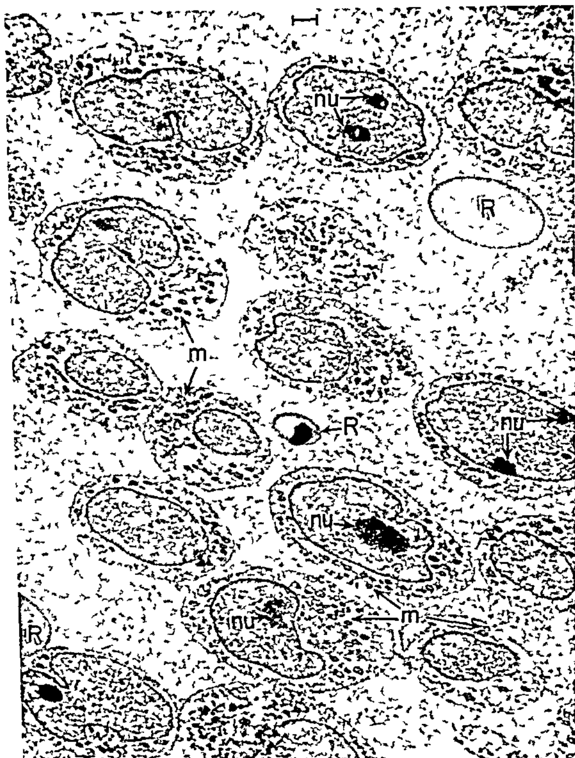
emia


MB
R
nu
MB
L
R
nu
L
MB(I)
nu
MB
MB
MB
nu
R
R
MB
nu
L
MB
MB
n

(Case 34a before treatment) 6900 X

Cytology of monoblasts With the exception of erythrocytes (*R*) all the cells in this field are monoblasts The cytoplasm contains numerous small mitochondria (*m*) which are mostly oval or round The cyto plasmic density varies from cell to cell but is not a useful criterion in determining cell age The nucleoplasm of the monoblast is of fairly uniform density except for the very dense nucleoli (*nu*) Two distinct densities of undifferentiated nucleoplasm are not developed until the cell approaches a maturity not present to a marked degree in any of the cells in this field These cells should be compared with those in the fields illustrated on pages 285 291 295 and 301 which include both early and late forms

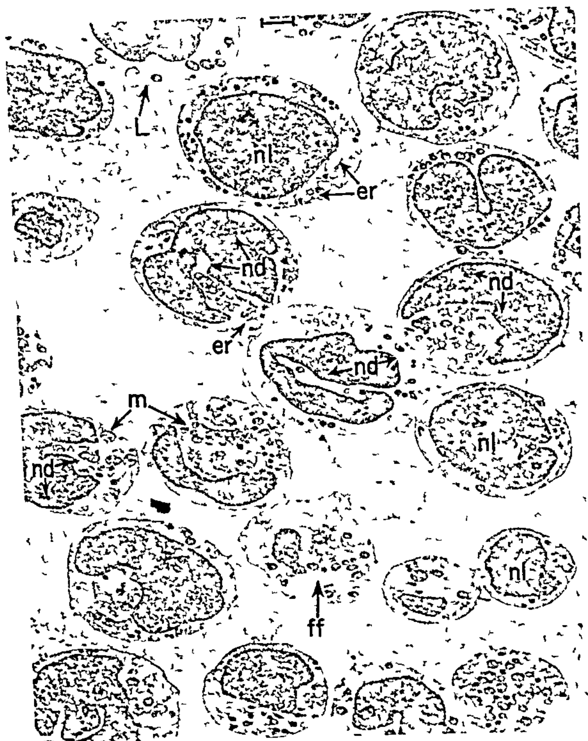
emia


nu
R
m
R
nu
nu
nu
m
R

(*Case 35b after treatment*) 9000 X

Group of monoblasts Most of them appear to be in the middle stage of development This is indicated chiefly by the nucleoplasmic densities which although fairly uniform throughout show definite indication of both light (*nl*) and dark (*nd*) areas In the course of differentiation the dark areas first appear close to the nuclear membrane and later are found in irregular clumps Some of these extend into the nucleus Most of the dark nucleoplasm is close to the nuclear membrane in these cells but there are some centrally located clumps Numerous mitochondria (*m*) are present in all the cells Endoplasmic reticulum (*er*) is conspicuous in some and scarcely visible in others One of the cells at lower center possesses a distinct fibrillar formation (*ff*) At top left is a lymphocyte (*L*) the cytoplasm of which is very light and contrasts with that of the monoblasts

emia


L
nl
er
nd
nd
er
nd
m
nd
nl
nl
ff

(Case 34a before treatment) 1100 X

Monoblasts with Auer bodies (*ab*) Note the light nucleolus (*nu*) A lymphocyte (*L*) is also present

(Case 34a before treatment) 7100 X

Typical monoblasts (*MB*) Except for the one at *MB*(*l*) they appear to be in the middle stage of development with little tendency for dark nucleoplasm to collect under the nuclear membrane An Auer body (*ab*) is visible in the cytoplasm of one of them The nucleoli (*nu*) are characteristically darker than the surrounding nucleoplasm The reverse is true in light micrographs as illustrated above The cell partially visible at the top of the field is a neutrophil (*N*) and its nuclear densities are characteristic (pages 36 to 47)


N
nu
M
MB
ab
MB
nu
MB
MB(I)

(Case 34a before treatment) *8100 X*

Monoblasts (MB) and lymphocytes (L) The nucleoplasm (*n*) of the lymphocytes is much denser than that of the monoblasts The lympho cytic cytoplasm is in general less dense than that of the monoblasts and contains fewer mitochondria The relative nuclear densities are useful criteria for distinguishing between these cell types But comparisons of cells should be restricted to a single field since the range of densities may vary considerably among different specimens The cell at *MB(l)* is nearly mature as indicated by the dense nucleoplasm (*nd*) collected under the nuclear membrane and even extending in clumps deep into the nucleus

nia

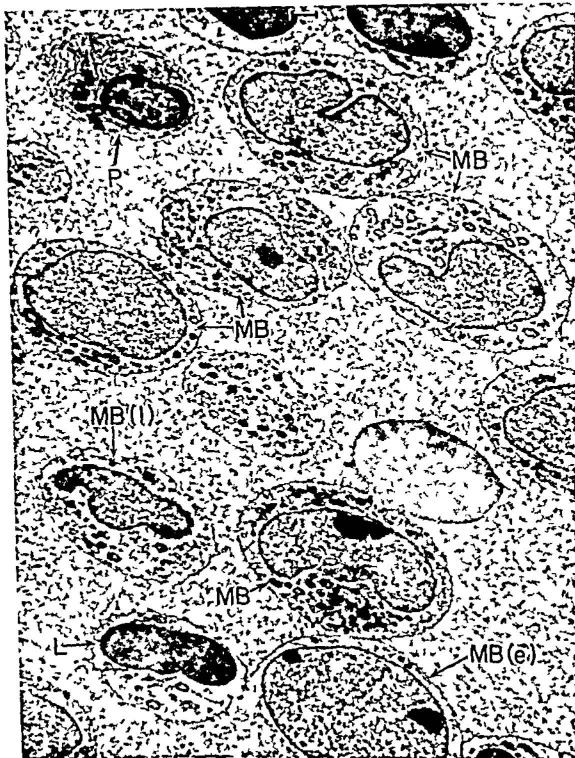


L
L
MB
MB
MB
MB(I)
MB

(Case 34a before treatment) 8100 X

Monoblasts (MB) and lymphocytes (L) The nucleoplasm (*n*) of the lymphocytes is much denser than that of the monoblasts The lympho cytic cytoplasm is in general less dense than that of the monoblasts and contains fewer mitochondria The relative nuclear densities are useful criteria for distinguishing between these cell types But comparisons of cells should be restricted to a single field since the range of densities may vary considerably among different specimens The cell at *MB(l)* is nearly mature as indicated by the dense nucleoplasm (*nd*) collected under the nuclear membrane and even extending in clumps deep into the nucleus

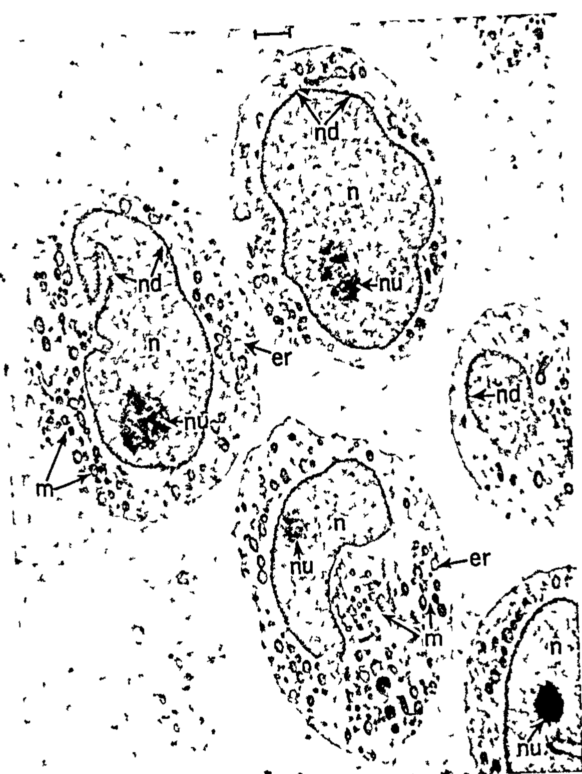
mia


P
MB
MB
MB(l)
MB
L
MB(e)

(Case 34a before treatment) 7100 X

Multiple cell types This field contains monoblasts (*MB*) a lymphocyte (*L*) and a plasma cell (*P*) The large oval nucleus light nucleoplasm and sparse cytoplasm of the cell at *MB(e)* suggest an early form The remainder of the monoblasts are apparently in their middle stage of development except possibly the one at *MB(l)* which shows a greater density of nucleoplasm near the nuclear membrane The distinction be tween lymphocytes and monoblasts is given on page 292 Plasma cells are described in greater detail elsewhere (pages 315 316 and 318 to 333)

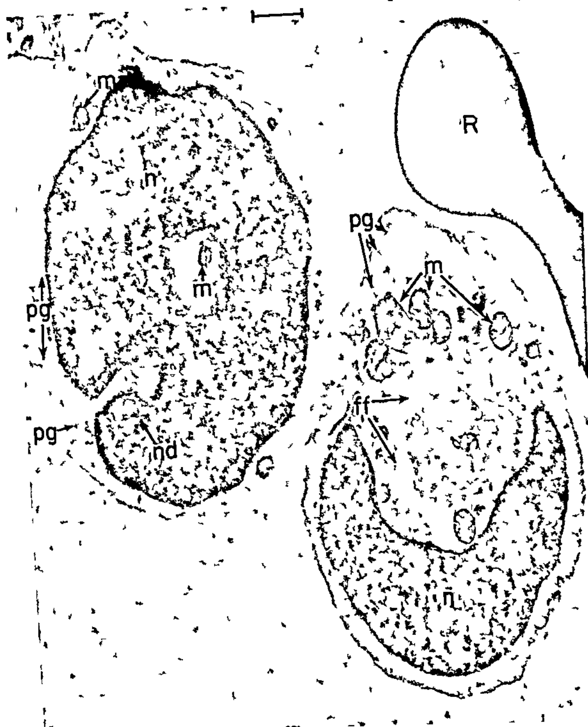
emia


nd
n
nu
nd
n
er
nu
m
nd
n
nu
er
m
n
nu

(Case 34a before treatment) 9300 X

Early forms The cells in this field are monoblasts whose structural features suggest a largely undifferentiated condition This is evident chiefly in the nuclei whose nucleoplasm (*n*) has a fairly even density throughout There is only faint suggestion of dark nucleoplasm (*nd*) and this is all close to the nuclear membrane The development of these areas represents the first recognizable step in nuclear differentiation Note the conspicuously dense nucleoli (*nu*) The cytoplasm of these cells contains typically abundant mitochondria (*m*) most of which are small and well rounded The cytoplasm itself has a generally speckled appearance largely due to random scattering of Palade granules These are however too small (about 130 A) to resolve individually at this level of magnification There is some endoplasmic reticulum (*er*) present but it is not a conspicuous feature of these particular cells

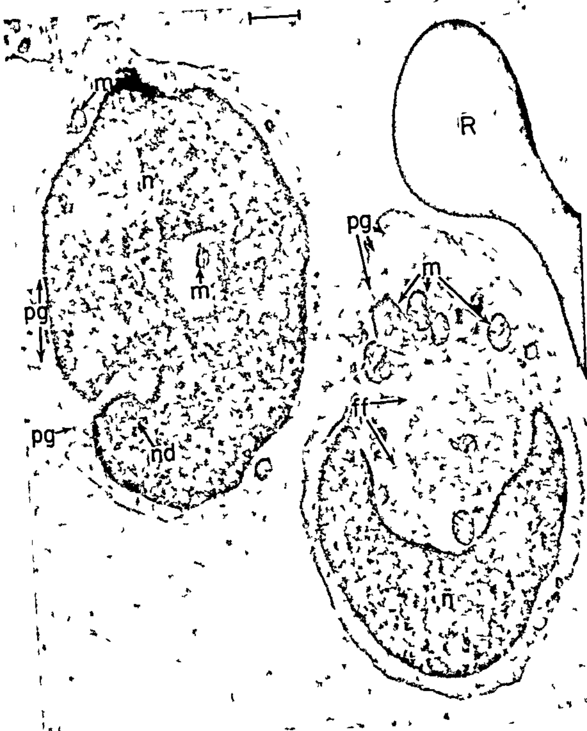
emia


m
R
n
pg
m
m
pg
pg
ff
pg
nd
n

(Case 35e after treatment) 14 000 X

Monoblasts The cytoplasm of the cell at upper left has a speckled ap pearance largely due to randomly scattered groups and clumps of Palade granules (*pg*) There is no discernible endoplasmic reticulum in this cell The nucleoplasm (*n*) has some suggestion of two densities The dark areas (*nd*) are located mainly subjacent to the nuclear membrane but some clumps are deep in the nucleus The oval area in the center of the nucleus is cytoplasm containing a mitochondrion (*m*) where the section passed through a nuclear indentation

The cell at lower right contains numerous mitochondria and Palade granules Within the curvature of the nucleus there is a faintly recogniza ble fibrillar formation (*ff*) (see pages 309 and 311) Note that the cyto plasm within the curvature of the nucleus and the fibrillar formation differs in texture from the remainder of the cytoplasm This difference is more striking in the cell illustrated on page 309 The nucleoplasm presents no special features and is comparable to that of the other cell An erythrocyte (*R*) is present


m
R
n
m
pg
pg
m
pg
nd
ff
n

GENERAL FIELDS

(Case 34a before treatment) **13 000 X**

Monoblasts in distinctly different stages of differentiation The lower one at $MB(e)$ has a fairly even nuclear density without marked concentration of dense nucleoplasm near the nuclear membrane It represents an early stage of development The one above at $MB(l)$ has both light (nl) and dark (nd) nuclear densities As is characteristic of maturing nucleoplasm the greater density is collected chiefly close to the nuclear membrane Although the cytoplasm of these two cells differs in detail there are no cytoplasmic features in either cell useful in judging the stage of development

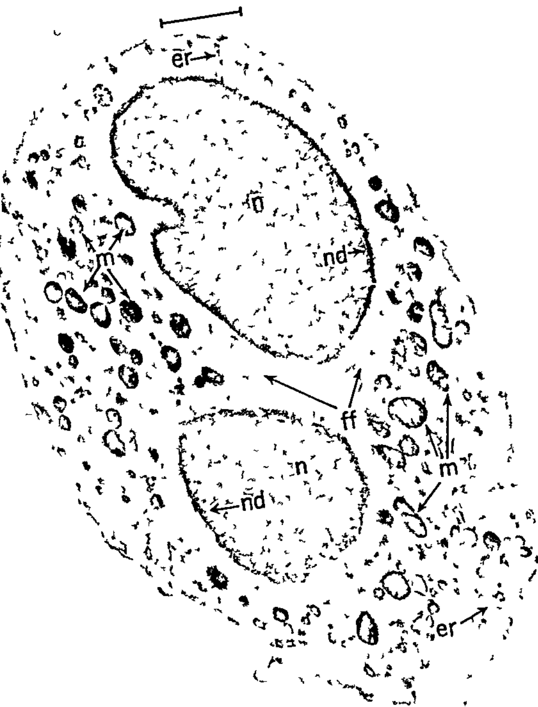

MB(l)
nd
nd
nt
MB(e)

GENERAL FIELDS

(Case 34a before treatment) 13 000 X

Monoblasts in distinctly different stages of differentiation The lower one at $MB(e)$ has a fairly even nuclear density without marked con centration of dense nucleoplasm near the nuclear membrane It represents an early stage of development The one above at $MB(l)$ has both light (nl) and dark (nd) nuclear densities As is characteristic of maturing nucleoplasm the greater density is collected chiefly close to the nuclear membrane Although the cytoplasm of these two cells differs in detail there are no cytoplasmic features in either cell useful in judging the stage of development

nia


er
m
n
nd
ff
n
nd
m
er

(Case 34a before treatment) 1100 X
Typical monoblast If a thin section passed through the cell in the direction indicated by the arrow two lobes of the nucleus would appear in the section but the Auer bodies (*ab*) and the nucleoli (*nu*) would not be present This would result in essentially the appearance on the facing page

(Case 34a before treatment) 22 000 X
Structural features of monoblast The cytoplasm is well populated with mitochondria (*m*) most of which are round or oval None exceeds 0 6 μ in length and most measure 0 2 to 0 3 μ Profiles of endoplasmic reticulum (*er*) are present but they are small and are not conspicuous The remainder of the cytoplasm is generally speckled its over all appearance resembling that of a mature monocyte At *ff* there is a faint but definite fibrillar formation This feature of the monoblasts in this type of leukemia is described in greater detail on pages 308 and 310

The nucleoplasm (*n*) has largely a single density without definite patterning into light and dark areas There is however some indication of a darker nucleoplasm (*nd*) near the nuclear membrane This represents the first recognizable approach to the mature nuclear pattern


ab
nd

(Case 34a before treatment) 1100 X
Monoblast containing Auer body (ab) Its rod like shape corresponds to the lower right illustration on the facing page

Ultrastructure of Auer bodies

Upper (Case 34a before treatment) 16 000 X
This cell is a typical monoblast containing an Auer body *(ab)* in the cytoplasm This particular one is probably cut in cross section as the Auer bodies are more likely to be rod shaped The remainder of the cell has typical features The generally even density of the nucleoplasm with only faint suggestion of darker material *(nd)* at the nuclear membrane indicates a fairly early developmental stage

Lower left (Case 34a before treatment) 27 000 X
This Auer body which is about 3 2 μ long is unusual for the swelling about 0 75 μ wide along its extent The shape of Auer bodies although most often rodlike is subject to considerable variation Note that there is some indication of internal ultrastructure in the form of a longitudinal rod of slightly greater density than the remainder of the structure It does not participate in the lateral swelling and is about 0 15 μ wide

Lower right (Case 34a before treatment) 41 000 X
The rodlike shape of this Auer body is typical It is about 0 4 μ wide and at least 2 5 μ long but since the lower end apparently passes out of the plane of section this measurement is probably short There is some indication of linear internal ultrastructure The considerable variation in density is probably a sectioning artifact Numerous Auer bodies appear to have some internal ultrastructure but its form is not constant and it *is never very clear Such structural organizations if they exist are very* vague



go
ab1
m
ab2
ab
n
0.1μ
n
ab
ab
n
m
g
1

Ultrastructure of Auer bodies

Upper left (Case 34a before treatment) **27 000 X**

The large oval Auer body above (ab_1) is characteristic in that although it shows some signs of internal structure the contrasts within it are not high and the organization is poor Portions of it are faintly speckled suggesting granules There is a linear formation extending longitudinally through the center but this is streaky and indefinite The smaller Auer body (ab_2) shows essentially the same characteristics Numerous mitochondria (m) and a Golgi zone (go) are present

Upper right (Case 34a before treatment) **27 000 X**

This Auer body (ab) is smooth and regularly oval and seems to be surrounded by a poorly organized membrane There is an irregular inclusion of slightly greater density within it and faint suggestion of granularity A portion of the nucleus (n) is present

Lower left (Case 34a before treatment) **67 000 X**

The Auer body (ab) occupying most of the field measures about 0 35 by 1 35 μ The small granule at g has comparable density and its margins resemble those of the Auer body It is about 0 1 μ wide Note the mitochondria (m) and a portion of the nucleus (n)

Lower right (Case 34a before treatment) **28 000 X**

This elongate Auer body measures about 0 4 by 1 8 μ The direction of cut during sectioning was upward to the left This accounts for the very dense margin along the lower right extent of the Auer body which is an indication of hardness A large portion of the nucleus (n) is visible


m
go
nd
n
nd
ff

FIBRILLAR FORMATION (CRESCENTIC AREAS)

Monoblast with highly developed fibrillar formation Structures of this sort have been described (see page 282) and called crescentic areas but this name has not been adopted because they are only occasionally seen as crescents Their actual configuration in sections depends on the haphazard plane of the cut with the result that no particular shape predominates Since they are made up chiefly of fine fibrils the name *fibrillar formation* seems to be appropriate

Upper (Case 35c after treatment) **18 000 X**

The oval fibrillar formation is located in a nuclear indentation Note that the cytoplasm within the oval is different from that of the rest of the cell The cell otherwise presents no unusual features having typical mitochondria (*m*) and Golgi zone (*go*) The nucleoplasm (*n*) is essentially of uniform density with only a very thin rim of darker material (*nd*) inside the nuclear membrane

Lower (Case 35c after treatment) **38 000 X**

This micrograph illustrates detail from the cell above The oval fibrillar formation can be observed to be made up of very fine fibrils which are about 75 A in diameter The open center of the oval is filled with granules with an average diameter of about 450 A Their density is variable and some of them appear to have light centers The appearance of this fibrillar formation and its core may be compared with another formation illustrated on page 299 Variations of typical fibrillar formations are illustrated also on pages 151 165 211 231 303 and 311

mia

m
go
nd
n
nd

Variations of fibrillar formation

Upper left (Case 34a before treatment) **20 000 X**
The fibrillar formation (*ff*) is present in the form of a nearly complete oval Note that a nuclear lobe (*n*) and two mitochondria (*m*) are enclosed within the oval The cytoplasm here does not appear to differ from the areas outside the oval Compare with the situations illustrated on pages 299 and 309

Upper right (Case 35c after treatment) **18 000 X**
The fibrillar formation (*ff*) in this particular field happens to be crescent shaped The enclosed area of cytoplasm shows no special features

Lower left (Case 35c after treatment) **27 000 X**
A small somewhat curved fibrillar formation (*ff*) is visible here Note that the curvature of the crescent is such that the oval could not be completed because of the proximity of the nucleus

Lower right (Case 35a before treatment) **27 000 X**
The very small fibrillar formation (*ff*) illustrated here represents about the least recognizable expression of this structure A similar minimal expression of a fibrillar formation may be observed in the cell illustrated on page 303


m
n
n
ff
ff
ff
ff

Features of monoblastic cytoplasm

Upper left (Case 34a before treatment) 50 000 X

The mitochondria (*m*) in this field do not show any special features Also present are some endoplasmic reticulum (*er*) and Palade granules (*pg*) All these structures appear to be normal

Upper right (Case 34a before treatment) 38 000 X

The mitochondria (*m*) and Golgi zone (*go*) in this field are typical as is the portion of the nucleus (*n*) that is visible There are numerous dark granules (*g*) in the mitochondrial matrices but these are found in the mitochondria of normal cells (pages 102 to 107)

Lower left (Case 34a before treatment) 38 000 X

The large oval mass with an irregular clear center is not identified It measures about 0 5 by 0 85 μ and is surrounded by a dark membrane Two smaller granules (*g*) each about 0 25 μ in the greatest diameter are also surrounded by a dense membrane and have a comparable matrix Mitochondria (*m*) Palade granules (*pg*) and a portion of the nucleus (*n*) are present

Lower right (Case 34a before treatment) 40 000 X

The oval body with contained vacuoles is not identified It measures about 0 45 by 0 75 μ The mitochondria (*m*) present no unusual features except some very light areas in their matrices A portion of the nucleus (*n*) is present This granule may be compared with those of normal blood cells on page 123


er
m
pg
n
go
m
m
g
n
g
pg
g
m
m
n
m

Features of monoblastic cytoplasm

Upper left (Case 34a before treatment) — 50 000 X

The mitochondria (*m*) in this field do not show any special features Also present are some endoplasmic reticulum (*er*) and Palade granules (*pg*) All these structures appear to be normal

Upper right (Case 34a before treatment) — 38 000 X

The mitochondria (*m*) and Golgi zone (*go*) in this field are typical as is the portion of the nucleus (*n*) that is visible There are numerous dark granules (*g*) in the mitochondrial matrices but these are found in the mitochondria of normal cells (pages 102 to 107)

Lower left (Case 34a before treatment) — 38 000 X

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Lower right (Case 34a before treatment) — 40 000 X

The oval body with contained vacuoles is not identified It measures about 0 45 by 0 75 μ The mitochondria (*m*) present no unusual features except some very light areas in their matrices A portion of the nucleus (*n*) is present This granule may be compared with those of normal blood cells on page 123

mia



PLASMA CELLS

This section on plasma cells is included because this cell type occurs both in normal blood (although rarely) and in eosinophilia One illus tration (page 323) is that of a plasma cell found in a stem cell leukemia The condition known as multiple myeloma in which plasma cells are released into the peripheral blood is sometimes called plasma cell leu kemia

The plasma cells constitute a cell type which is conspicuously dif ferent in electron microscopy from all other normal and leukemic cell types because of the extensive development of the endoplasmic reticu lum In light microscopy plasma cells are deceptively similar to lympho cytes because the endoplasmic reticulum is too fine to be clearly dis tinguished and the other characteristics are much the same But in elec tron microscopy where the endoplasmic reticulum is readily identified there is no confusion between lymphocytes and plasma cells

In the following descriptions the area near the nucleus not containing endoplasmic reticulum which corresponds to the light area in the same place in light microscopy is given the nonspecific name *paranuclear area* This name was chosen because the area does not conform struc turally to any known element of electron microscopy with any degree of consistency More often than not it appears to be a Golgi zone but frequently its structure does not conform to the classic Golgi zone pattern



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Plasma cells have been described in electron microscopy by Bernhard and Leplus [1] Braunsteiner and Pakesch [2] Braunsteiner et al [3] and Policard et al [4]

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1 Bernhard W and R Leplus La methode de coupes ultrafines et son application a l etude de l ultrastructure des cellules sanguines *J suisse med* 85 1–10 1955

2 Braunsteiner H and F Pakesch Electron microscopy and the functional significance of a new cellular structure in plasmocytes A review *J Hemat* 10(6) 650–654 1955

3 Braunsteiner H K Fellinger and F Pakesch Electronenmikroskopische Untersuchungen uber Zell struktur und Zell funktion *Klin Woch* 33 4-8 1955

4 Policard A A Collet and L Giltaire Raylte Étude au microscope electronique de l action de poussieres de silice sur les plasmocytes et les cellules reticulohistiocytaires des mammiferes *Rev hemat* 9 403–418 1954

PLASMA CELL MICROGRAPHS

(Multiple Myeloma Case 36 before treatment) 1600 X

Plasma cell from Wright stained peripheral blood smear The light paranuclear area (*pna*) corresponds to the same structure in the electron micrograph (facing page upper) The faint light speckling of the cytoplasm probably corresponds to the endoplasmic reticulum so conspicuous in the electron micrographs

Structural features of plasma cells

Upper (Multiple Myeloma Case 36 before treatment) 16 000 X

The eccentrically located nucleus has well differentiated nucleoplasm with light (*nl*) and dark (*nd*) areas The paranuclear area (*pna*) in this cell is characteristic Portions of it appear to be coarsely granular but there are numerous small flattened sacs which are typical of the Golgi zone (*go*) The endoplasmic reticulum (*er*) is characteristically plentiful in the form of cisternae Mitochondria (*m*) are numerous Some of them seem to be suspended in a sac of endoplasmic reticulum (m_1) but are really surrounded by the membrane of the endoplasmic reticulum which forms open communication with the rest of the cytoplasm in the manner of a mesentery (m_2) Thus the mitochondria are really intracytoplasmic

Lower (Multiple Myeloma Case 36 before treatment) 16 000 X

The nucleus of this plasma cell is typical It is eccentric and oval and has well differentiated nucleoplasm (*nl nd*) The paranuclear area which is so conspicuous in the cell above is not included in the plane of section The cisternae of endoplasmic reticulum (*er*) and the mitochondria (*m*) are visible


m1
m2
pna
go
er
er
m
e
nl
nd
m
er
nd
nd
er
nl

(Multiple Myeloma Case 36 before treatment) 19 000 X

Plasma cell of unusual shape The outstanding structural feature of this cell type is the abundant endoplasmic reticulum which may be in the form of elongate flattened sacs (*er1*) or circular or oval profiles (*er2*) Palade granules (*pg*) occasionally give the membranes of the endo plasmic reticulum a beaded appearance Mitochondria (*m*) are very numerous and tend to take a rod shaped form but may have complex outlines (*m1*) They are about 0 25 μ wide and may reach a length up to 2 μ The nucleus has no clearly distinguishing features The nucleo plasm however may be recognized as differentiated into light (*nl*) and dark (*nd*) areas The latter are gathered chiefly near the nuclear mem brane but may extend freely into the interior The interior of the endo plasmic reticulum in these cells is largely clear (*er1*) but has a faint gray density in some areas (*er2*)


er 2
m 1
pg
nd
er 1
nl
m

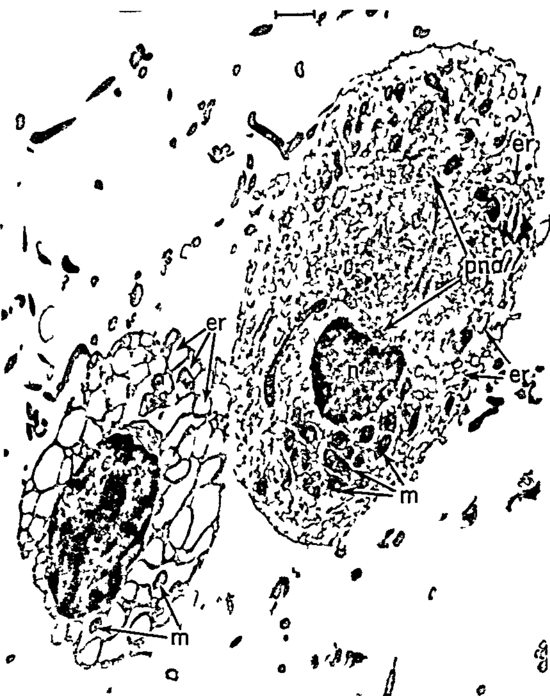
(Stem Cell Leukemia Case 22 during treatment) 28 000 X

Typical plasma cell Because of the eccentricity of the nucleus these cells are frequently sectioned in a plane in which the nucleus is not represented The most conspicuous feature of plasma cells in electron microscopy is the extraordinary development of the endoplasmic reticulum (*er*) which fills the greater part of the cytoplasm in the form of irregularly flattened sacs of large size In the undifferentiated cytoplasm around the sacs numerous Palade granules (*pg*) are found often arranged along the border of the sacs Numerous large mitochondria (*m*) are present The paranuclear area that is pale in light microscopy and which contains the cytocentrum and the Golgi zone is bracketed by arrows in this micrograph (*pna*) It presents an appearance typical of the Golgi zone In this particular cell the interior of the sacs of endoplasmic reticulum is not clear but contains a light gray matrix


pna
m
pg
er

(Multiple Myeloma Case 36 before treatment) 10 000 X

Variations of structural organization The larger cell has the more normal appearance of the two There are recognizable mitochondria (*m*) endoplasmic reticulum (*er*) a paranuclear area (*pna*) and a nucleus (*n*) The more compact appearance is largely due to the fact that the endoplasmic reticulum is neither extensive nor swollen The smaller cell is chiefly characterized by the dilated sacs of endoplasmic reticulum Mitochondria can be recognized in the sparse areas of cytoplasmic matrix that are present The nucleus appears to be in good condition


er
pnd
er
n
er
m
m

Upper (Multiple Myeloma Case 36 before treatment) 14 000 X
Typical features of plasma cells The endoplasmic reticulum (*er*) is con spicuous both as sacs and as circular profiles some having a light gray matrix while others are clear Mitochondria (*m*) are numerous The paranuclear area (*pna*) is evident but does not show the usual charac teristics of a Golgi zone so clearly as in some cells (pages 319 and 329) The nucleus is eccentric with well differentiated nucleoplasm (*nl n l*) although the contrasts are not high

Lower (Multiple Myeloma Case 36 before treatment) 23 000 X
Non nucleated section of plasma cell There are abundant endoplasmic reticulum (*er*) and many mitochondria (*m*) The paranuclear area (*pna*) is typical and shows the characteristics of the Golgi zone (*go*)


nl
nl
nd
m
pna
er
er
m
er
go
pna

Plasma cells with strongly dilated endoplasmic reticulum This condition is common in cells of multiple myeloma

Upper (Multiple Myeloma Case 36 before treatment) 15 000 X
The swollen sacs of endoplasmic reticulum (*er*) occupy most of the cytoplasmic area Most of them seem to contain a structureless slightly gray matrix which barely contrasts with the background outside the cell Mitochondria (*m*) are evident A considerable part of the paranuclear area (*pna*) is visible but the nucleus has escaped the plane of section

Lower (Multiple Myeloma Case 36 before treatment) 16 000 X
The sacs of endoplasmic reticulum (*er*) in this cell are swollen as in the cell above In only a few areas do they seem to have a structureless light gray matrix Mitochondria (*m*) are evident The paranuclear area (*pna*) is largely filled with the flattened sacs of the Golgi zone (*go*) The small portion of the nucleus present has well differentiated nucleoplasm (*nl nd*)


er
m
pna
er
er
er
m
pna
er
go
nd
nl
er

(Multiple Myeloma Case 36 before treatment) 27 000 X

Plasma cell with inclusions In multiple myeloma the plasma cells in the peripheral blood often contain very dense inclusions (*i*) which on close examination appear to contain internal ultrastructure resembling mitochondrial cristae The size of these inclusions usually conforms to mitochondrial dimensions Occasionally a circular form such as that at *c* is present which does not correspond to mitochondrial morphology The remainder of the cytoplasm is normal with numerous profiles of endoplasmic reticulum (*er*) and a large population of Palade granules (*pg*)


pg
o
er
l
er
er

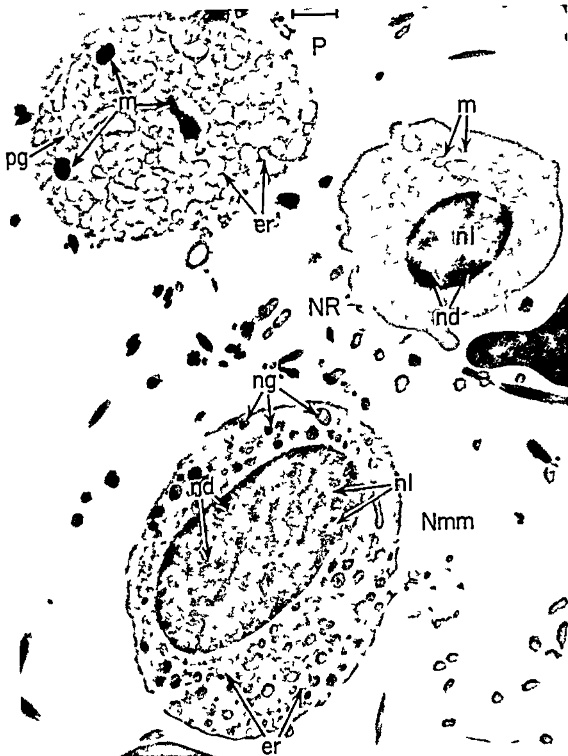
(Multiple Myeloma Case 36 before treatment) 12 000 X

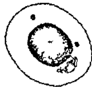
Mixed cell group This field contains a plasma cell (*P*) a nucleated erythrocyte (*NR*) and an immature cell of the neutrophilic series (*Nmm*)

The cytoplasm of the plasma cell (*P*) is typically riddled with profiles of endoplasmic reticulum (*er*) which in this section happen to be in circular or oval profiles Palade granules (*pg*) can be seen in the undif ferentiated cytoplasm and lining the endoplasmic reticulum The three dark bodies are mitochondria (*m*) whose matrices are much denser than usual a common characteristic of mitochondria in the plasma cells of multiple myeloma

The nucleated erythrocyte (*NR*) is typical of the middle stage of the erythrocytic series with light (*nl*) and dark (*nd*) nucleoplasm The cytoplasm is of even but pronounced density and has very few inclusions (mitochondria *m*) Compare this cytoplasmic density with that of the two other cells

The remaining cell (*Nmm*) is obviously a member of the neutrophilic series because of the numerous specific granules (*ng*) The number of granules the occasional large profiles of endoplasmic reticulum (*er*) the nuclear size and shape and the clear nucleoplasmic differentiation all indicate a neutrophilic myelocyte A distinctly atypical feature is the mottled patternization of light and dark nucleoplasm which is unex plained


P
m
pg
er
m
nl
NR
nd
ng
nd
nl
Nmm
er



THE ERYTHROCYTIC SERIES

This short section illustrates cells in various stages of development in the erythrocytic series The fields are chosen to present to the reader the stages that may be expected to be observed in any of the leukemias of the foregoing pages No attempt is made to present a comprehensive series of development

In the myeloblastic leukemias very early stages predominate Here the greatest difficulty is experienced in identifying members of the series Often the only satisfactory criterion is the darker shade of both cytoplasm and nucleus of the early erythroblasts as compared to neighboring myeloblasts However a few later stages more readily recognizable are present Some of these are present in fields with other cells on pages 161 203 207 215 247 and 249

Numerous erythrocytic forms are present in the peripheral blood of multiple myeloma The two examples illustrated (page 341) were chosen because their development is intermediate between the earliest forms and the middle stages characteristic of granulocytic leukemia

The granulocytic leukemias have cells much further along in the erythrocytic series of development They are readily distinguishable from all other cell types A single typical example is illustrated on page 343

ERYTHROCYTIC SERIES MICROGRAPHS

Earliest recognizable cells of erythrocytic series

Upper (Myeloblastic Leukemia Case 20 before treatment) 19 000 X
The transition from myeloblast to the erythrocytic series can first be detected by a fairly even increase in the density of the undifferentiated cytoplasm At this stage there are no perceptible changes in the formed elements or the nucleus The illustrated cell represents this stage in which the mitochondria (*m*) and the endoplasmic reticulum (*er*) are seen against a background of undifferentiated cytoplasm that is some what darker than usual The nucleoplasm appears to have light (*nl*) and dark (*nd*) areas that are more definite than in myeloblasts The *irregular contour of this cell is typical of the stage*

Lower (Myeloblastic Leukemia Case 20 before treatment) 15 000 X
This cell is somewhat more advanced in its development in the erythro cytic series than the one above The full complement of formed elements such as mitochondria (*m*) and endoplasmic reticulum (*er*) is present in the cytoplasm but the undifferentiated cytoplasm is darker Its density may be compared with that of the cytoplasm of the small portion of the cell directly above it The nucleoplasmic differentiation has progressed so that definite areas of light (*nl*) and dark (*nd*) nucleoplasm are visible *the latter collected chiefly inside the nuclear membrane*

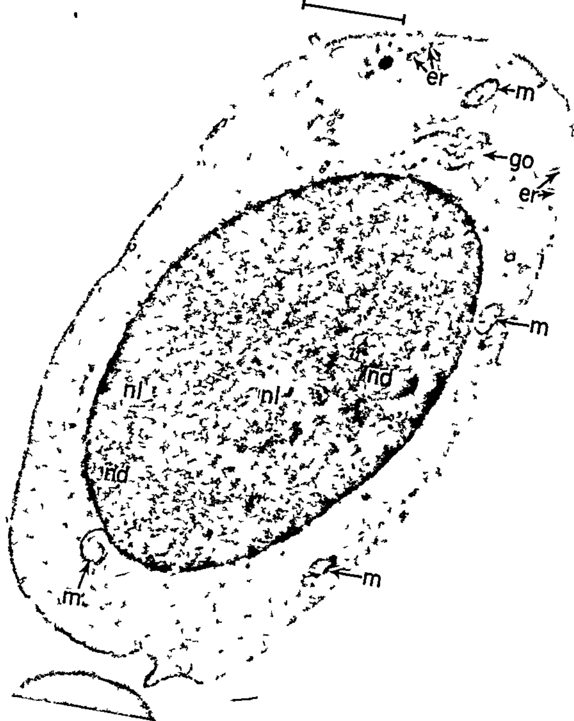
nl
nd
nl
nd
m
go
er
m
er

Developing erythroblasts

Upper (Multiple Myeloma Case 36 before treatment) 17 000 X
The formed cytoplasmic elements in this cell are mostly collected beneath the nucleus but the remainder of the cytoplasm is clear The nucleo plasmic differentiation is very well developed with light (*nl*) and dark (*nd*) areas in high contrast with each other The entire cell has an irregular outline

Lower (Multiple Myeloma Case 36 before treatment) 17 000 X
This nucleated erythrocyte appears to be in about the same stage as the one above Fairly large areas of the cytoplasm are practically free of formed elements but elsewhere there are mitochondria (*m*) endoplasmic reticulum (*er*) and the Golgi zone (*go*) The two nucleoplasmic densi ties (*nl nd*) are well established

ries


er
m
go
er
m
nd
nl
nl
nd
m
m

(Granulocytic Leukemia Case 15 before treatment) 27 000 X

Nucleated red blood cell The cytoplasm is of fairly high density and is quite even Most of the formed elements have been lost but those re maining are recognizable as mitochondria (*m*) a few small profiles of endoplasmic reticulum (*er*) and what appears to be the remnants of the Golgi zone (*go*) The nucleus is of smooth contour and the nucleo-plasm is well differentiated into light (*nl*) and dark (*nd*) areas All the characteristics of this cell are typical

ries

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